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Conventional and Modern Methods for the Detection of Foodborne Pathogens of Livestock Origin

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ABSTRACT

Regarding recent advancements in preservation of food methods and safety protocols, substantial disease outbreaks associated with foodborne pathogens, including bacteria, viruses, parasites, and fungi, signify that these pathogens remain considerable threats to public health. Despite the existence of comprehensive evaluations on methods of foodborne pathogens detection, the majority predominantly focus on bacteria, overlooking the growing significance of other pathogens, including viruses. This review article offers a thorough exploration of methods for detecting foodborne pathogens, with a particular focus on pathogenic bacteria, viruses, parasites, and fungi. This review focuses on several contemporary approaches that are available for the identification of existing and developing foodborne as culture-based methods, microscopical examination for foodborne parasites, polymerase chain reaction (PCR), real-time polymerase chain reaction (RT-PCR), MALDI-TOF-MS, immunoassays, next-generation sequencing (NGS) methods, nanobiotechnology, biosensor, and DNA microarray. This indicates that the comprehensive use of these techniques can facilitate early diagnosis and management of foodborne infections, hence improving public health and decreasing the incidence of outbreaks.

Key words: Foodborne pathogens; Detection; PCR and NGS methods; Rapid pathogen diagnostics; Public health surveillance

INTRODUCTION

Food-producing animals serve as major reservoirs for numerous foodborne pathogens that pose significant public health risks globally (Heredia and García 2018). Food-borne zoonotic bacterial pathogens of livestock origin cause the most common human intestinal diseases in many countries (Thorns 2000). These zoonotic bacteria are transmitted primarily through the consumption of raw animal products or foods processed under unsanitary conditions and eggs from infected animals (Zenu and Bekele 2024).

Normal flora, usually bacteria, in the gastrointestinal tract and skin of humans are harmless and help the body. However, many parasites, viruses, fungi, and bacteria are dangerous (Aladhadh 2023). The most common foodborne pathogens of public health concern are bacteria. However, because they are also known to cause outbreaks of foodborne illnesses, other pathogenic groups, such as viruses and fungi, are important (Aladhadh 2023). Microorganisms have a crucial role in food safety, production, and spoilage. The interactions of food with microbes may render it inappropriate for human consumption (Iulietto et al. 2015; Saucier 2016). Some foodborne microbes are pathogenic and lead to food spoiling, providing hazards to the safety of food and contributing to foodborne illnesses among food handlers, customers, and society in general (Al-Zaidi et al. 2022). Foodborne illnesses and diseases constitute significant hazards to the health of humans and life, with over 200 recognized foodborne infections. Prevalent foodborne pathogens comprise microorganisms such as *Bacillus cereus*, *Clostridium botulinum*, *Campylobacter* species, *Acinetobacter* spp. *Clostridium perfringens*, *Clostridium difficile*. Certain serogroups of *Escherichia coli*, especially *O157:H7*, *Listeria monocytogenes*, *Salmonella* species, *Klebsiella* species, *Shigella* species, *Yersinia pestis*, *Staphylococcus aureus* and *Vibrio* species. Foodborne fungus species, including Penicillium, Claviceps, Aspergillus, and Fusarium, pose a public health issue due to their mycotoxin production (Martinović et al. 2016). Rotavirus, Hepatitis A and E viruses, Norovirus, Enterovirus, Astrovirus, Sapovirus, Achivirus, and Adenovirus are among the most common foodborne pathogenic viruses (Pexara et al. 2020; Lianou et al. 2023). The majority of detection is needed in the fields of

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pharmaceuticals, food, water, biodefense, public health, and the environment (Khan et al. 2010).

The potential for parasites to cause foodborne diseases has been realized in recent decades. *Toxoplasma, Giardia, Cyclospora,* and *Cryptosporidium* species are the most common parasites concerning food worldwide. Even though food can transmit other parasite species such as *Taenia* and *Trichnella* spp. (Dawson 2005), When compared to bacteria and viruses, parasites have been the most ignored group of pathogens. Many parasitic infections do not manifest as acute diseases but instead affect their hosts in a chronic, long-term manner, besides that the parasites are frequently linked to poverty is another factor contributing to their neglect. Populations most at risk for parasitic infections reside in areas with insufficient or nonexistent basic infrastructure, such as housing, transportation, sanitation and water supply (Robertson 2018).

Methods of detection were established for pathogens to guarantee adherence to established regulatory and legal food safety standards depending on the presence or absence of pathogens and their numbers (Aladhadh 2023). Numerous methodologies have been developed for foodborne pathogen identification (Priyanka et al. 2016). Food preservation methods that prolong shelf life and eliminate spoilage and pathogens have improved recently. Refrigerating and freezing, using chemical preservatives such as essential oils and bacteriocins and vacuum or changed environment packaging are common approaches. However, previous studies suggest environmental or food preservation stress may allow some pathogens to persist as metabolically compromised cells with reduced metabolic capabilities. Pathogens may recover from irreversible or reversible damage (Aladhadh 2023). Consequently, rapid detection is required to prevent the dissemination of the infectious agent before the beginning of a significant outbreak (Priyanka et al. 2016).

Foodborne microorganisms still present significant health threats to the public, affecting food production and safety. Culture media, nucleic acid sequence-based amplification (NASBA), DNA microarray, polymerase chain reactions (PCR) such multiplex PCR, real-time PCR, reverse transcriptase PCR (RT-PCR), and next-generation sequencing (NGS), fluorescence-based, colorimetric, and electrical biosensors, as well as immunological and nanotechnology-based techniques, have all been used in several studies on the detection of foodborne pathogens using both culture-dependent and independent methodologies (Law et al. 2015; Foddai et al. 2020). This review emphasizes molecular techniques such as PCR (End-point PCR, Multiplex PCR, RT PCR, etc.) and NGS to enhance our understanding of the identification of various foodborne pathogens, concerning the detection of foodborne parasites utilizing conventional microscopical, serologic, and molecular diagnostic techniques (Zolfaghari Emameh et al. 2018).

Foodborne illnesses

Due to underreporting and difficulties in establishing causal relationships between food contamination and subsequent sickness or mortality, the economic and public health effects of foodborne diseases have often been underestimated. The first assessments of the public health impact of illness burden concomitant to 31 foodborne pathogens such as bacteria, viruses, parasites, toxins, and chemicals at the global and sub-regional levels as shared in the 2015 WHO report. These findings provided insights at both global and regional levels. These estimates suggested that 420,000 deaths and more than 600 million cases of foodborne illnesses could occur each year. Foodborne diseases predominantly affect populations, particularly children under five, with the greatest impact observed in countries with low or middle incomes. The World Bank report from 2019 highlights the economic impact of foodborne diseases, estimating a total productivity loss of US$ 95.2 billion annually in low- and middle-income countries. Additionally, the annual cost of treating foodborne illnesses was valued at US$15 billion.

When a pathogen enters the body through food and then establishes itself often multiplying in the human host, foodborne disease results. As an alternative, it can happen when a toxigenic bacteria infiltrates a food product and creates a toxin that the human host consumes. Generally speaking, there are two primary categories of foodborne illness: Foodborne intoxication (b) and foodborne infection (a). In foodborne infections, the incubation period typically results in a longer duration from ingestion to the onset of symptoms compared to foodborne intoxications (Bintsis 2017). Foodborne pathogens including bacteria, viruses, parasites, and fungi, signifying that these pathogens remain considerable threats to public health (Fig. 1).

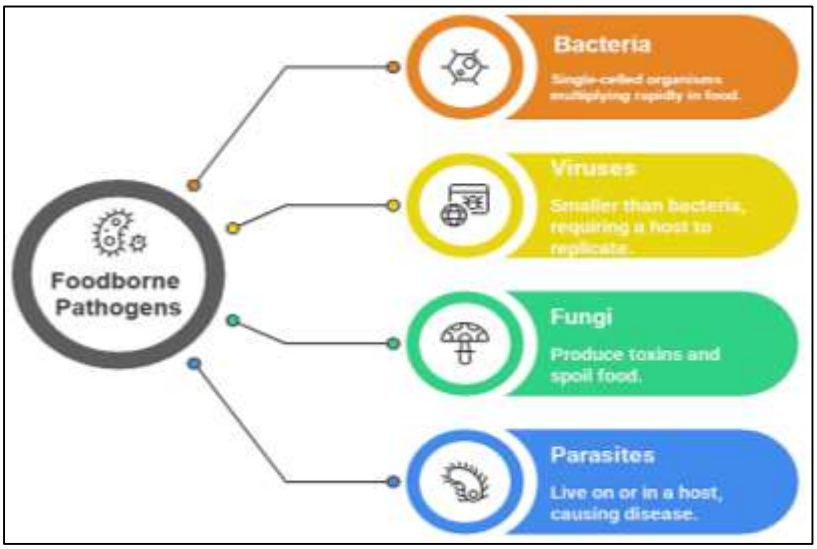

Fig. 1: Foodborne pathogens.

Incidences of foodborne diseases

Bacteria

Bacterial foodborne diseases are a worldwide concern. In the USA, the annual cost of food-related illnesses ranged from 128,000 to 325,000 hospitalizations, 3,000 to 5,000 fatalities, and at least 76 million illnesses, amounting to up to $83 billion (Mead et al. 1999; Nyachuba 2010). The Australian Health Department estimates that there are 4.1 million foodborne illnesses in Australia (Kirk et al. 2014). 20,017 human cases and 3,086 foodborne disease outbreaks were found in the European Union. In 2020, *Salmonella* was the most frequently identified bacterial agent responsible for these outbreaks. Along with Salmonella, Campylobacter, mainly derived from raw milk and undercooked poultry and Yersinia, Shiga toxin-producing *Escherichia coli* (STEC), Enterohaemorrhagic *E. coli* (EHEC) is mainly associated with unpasteurized milk, undercooked meat, and contaminated vegetables and fruits. A virulent subtype *E. coli* O157:H7 and Vibrio cholerae associated with through consumption of contaminated water or food (Fisheries 2007).

Along with *Salmonella*, *Campylobacter*, mainly derived from raw milk and undercooked poultry, and *Yersinia*, Shiga toxin-producing *Escherichia coli* (STEC), Enterohaemorrhagic *E. coli* (EHEC) is mainly associated with unpasteurized milk, undercooked meat and contaminated vegetables and fruit. A particularly dangerous strain, *E. coli* O157:H7, is both verotoxigenic and diarrhoeagenic. *Vibrio cholerae*, another harmful bacterium, spreads through contaminated water or food, (Fisheries 2007).

Listeria monocytogenes is considered a causal agent of foodborne infections in the EU in 2020. Illnesses associated with *L. monocytogenes* were among the leading causes of human deaths in the EU throughout this period (Le Marechal 2021). Globally, it is valued that approximately 3 million cases of diarrhea attributable to foodborne microbial infections occur yearly. Bacterial toxins constituted the second most frequent cause of illness in food- and waterborne outbreaks, responsible for 19.5% of reported outbreaks (Bintsis 2017).

Fungi

Outbreaks of diseases linked to fungal foodborne pathogens are few, unlike bacteria and viruses, as only approximately 300 of the detected 1.5 million species of fungi are recognized as harmful to humans. However, there are significant cases of disease outbreaks, mainly due to fungal secondary metabolites, such as toxins, some people especially those with weakened immune systems, like transplant recipients, or those undergoing immunosuppressive therapies. Nevertheless, rarely, some immunocompetent individuals may also become susceptible to foodborne fungal infections. In 2013, a gastroenteritis outbreak (characterized by vomiting, nausea, and diarrhea) impacted approximately 200 individuals in the USA who purchased yogurts contaminated with *Mucor circinelloides*. An epidemic of food poisoning caused by *Rhizopus microsporus* was documented in seven hospital patients in Hong Kong, attributed to contaminated ready-to-eat meals pre-packaged or cornstarch utilized in the production of allopurinol tablets. A previous review (Paterson et al. 2017) reported that certain filamentous fungi, including *Aspergillus*, *Fusarium*, and *Mucor*, can induce localized infections in immunocompetent individuals when ingested through contaminated food or inhaled.

Viruses

Hepatitis A and noroviruses are the main causes of foodborne diseases (Le Marechal 2021). According to earlier research, 20% of all outbreaks in the EU in 2014 were caused by viral agents. Enteroviruses cause meningitis/encephalitis, heart problems, and hand and foot infections, whereas foodborne hepatitis viruses cause hepatitis A or E. Noroviruses, Astroviruses and Rotaviruses cause gastroenteritis. They can spread through touch, droplets, or the fecal-oral route to fish, shellfish, oysters, and mollusks, as well as through undercooked and raw meat and liver-related Hepatitis (Pexara et al. 2020). According to recent reviews of documented virus-associated foodborne illness outbreaks, noroviruses were linked to the 2017 outbreak aboard a Royal Caribbean cruise line, the 2017 outbreak of gastroenteritis during the 2018 Winter Olympics in South Korea (194 cases) and the 2017 outbreak of frozen raspberry-related illness in Canada (700 cases) (Miranda et al. 2019). Major incidences are 162 Hepatitis A virus infections in the United States in 2011 (due to the ingestion of contaminated seeds of pomegranate) and more than 1100 cases detected in China in 2012 (Norovirus attributed to frozen strawberries). In 2020, West Nile virus-related diseases were among the predominant foodborne pathogens causing more human deaths in the EU (Le Marechal 2021).

Parasites

An estimated 407 million cases of human parasite disease occur worldwide each year, with foodborne illness responsible for 52,000 of these deaths (Torgerson et al. 2015). Humans can become infected by consuming raw or undercooked meat contaminated with the cyst stages of *Toxoplasma gondii*, *Sarcocystis* spp., *Trichinella* spp., and *Taenia* spp., the foodborne parasites of greatest concern globally (Dorny et al. 2009). Numerous parasites, such as trematodes (*Opisthorchis* spp., *Clonorchis sinensis*), nematodes (*Gnathostoma*, spp., Anisakidae) and cestodes (*Diphyllobothrium* spp., *Spirometra*), can infect humans through eating the meat of fish, amphibians, and reptiles (Dorny et al. 2009). Besides protozoan parasites (*Cryptosporidium*, *Cyclospora*, and *Giardia*) that have been revealed to cause foodborne and waterborne illnesses, on other hands, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) categorized A “top ten" important foodborne parasites in July 2014. Millions of people's health may be impacted by these parasites, which include *Echinococcus granulosus*, *Taenia solium*, *Echinococcus multilocularis*, *Toxoplasma gondii*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Trichinella* spp., *Opisthorchiidae*, *Ascaris lumbricoides*, and *Trypanosoma cruzi*. (Xiao et al. 2015; Zolfaghari Emameh et al. 2018).

Toxins

Mycotoxins, marine biotoxins, cyanogenic glycosides, and toxic mushrooms are a few types of natural poisons. Mold on grain produces mycotoxins like aflatoxin and

ochratoxin, which infect grain and maize. Prolonged exposure can impair normal growth, induce cancer, and damage the immune system. The human body and the environment produce persistent organic pollutants (POPs). Dioxins and polychlorinated biphenyls (PCBs) are among the waste products produced by industrial processes and trash incineration. They are common and build up in animal food systems (Fisheries 2007).

Detection of food pathogens

Before discussing many frequently utilized pathogen detection methods in recent literature, this review offers a succinct application of the principles defining each method category, including culture-based techniques, immunological assays, PCR, next-generation sequencing methodologies, and biosensors. Appropriate sampling and storage procedures must be followed for any of these techniques to be effective. The food sample type, the microbial groupings, and the microbial detection techniques all influence the sampling strategy. Authorized organizations including the FDA, FSIS/USDA, ISO, and AOAC have created standardized guidelines for sample collection and analysis for procedures involving microbial cultures isolation (Da Silva et al. 2018). Depending on the requirements given by the makers of the kit, reagent, and equipment, standardized procedures for sample collection, analysis, and data interpretation can be acquired.

Culture-based methods

Despite modern methods, culture-based methods have always been the primary techniques for foodborne pathogens (Chen et al. 2021; Patil-Joshi et al. 2021; Altayb et al. 2023). This approach provides a definitive indication of the existence of a certain pathogen, including bacteria and fungi. These are cheap, easy to use, and successful when targeting microorganism requirements for development are known and culture mediums are employed to enrich, selectively isolate, or distinguish them. For identification, cultures can be tested for colony characteristics, biochemical characterization, Gram staining reaction, MALDI TOF MS, and PCR-sequencing (Pexara et al. 2020). Despite its advantages, the primary disadvantage of the culture-based method is low sensitivity as not all microorganisms are culturable, resulting in the underestimation of foodborne pathogens. Its prolonged growth rate results in significant delays in obtaining the ultimate result, which may prove deleterious. All these media require 18-24 hours for bacteria and longer for fungi to provide accurate results, reflecting a prolonged turnaround time (Law et al. 2015). They might not be appropriate for the quick microorganisms detection on-site or the detection of real-time foodborne pathogens(Tietjen et al. 1995).

The culture of *E. coli* O157:H7 on the Sorbitol MacConkey agar (SMAC), which makes use of the sorbitol fermentation principle, is a well-known example that shows a high success rate and cost-effectiveness (Hirvonen et al. 2012). This method's main drawbacks are its lengthy turnaround time and the false-positive results, which are linked to the rise of sorbitol-fermenting non-O157 and O157 STEC20 serotypes (Hirvonen et al. 2012). By using a chromogenic medium for STEC isolation, the drawbacks of SMAC agar can be addressed while improving sensitivity and specificity. The main advantage of this is the improved color-based discrimination (Gouali et al. 2013). The medium is commonly referred to as CHROMagar due to the utilization of chromogenic material. Although it is relatively more successful than SMAC, a significant limitation is that CHROMagar lacks sensitivity to all strains (Hirvonen et al. 2012).

Cefsulodin-Irgasan-Novobiocin (CIN) was utilized to differentiate between *Yersinia enterocolitica* and non-*Y. enterocolitica*, which is a selective medium known for its exceptional capacity to identify bacterial species. A chromogenic substrate, fermentable sugar cellobiose, and a selective inhibitor that inhibits other microbes are all present in the *Y. enterocolitica* chromogenic medium (Tan et al. 2014). This reveals that the purple/blue colonies developed on CIN agar correspond to *Y. enterocolitica* and *Y. pseudotuberculosis*, which are significant foodborne pathogens responsible for yersiniosis and mainly cause food poisoning in tofu (Tan et al. 2014).

E. coli, *L. monocytogenes*, Yersinia, Salmonella, Campylobacter, Clostridium, Enterobacteria, and Bacillus species that can cause foodborne illnesses by both the bacteria themselves and their released toxins often cause Foodborne infections. These groups of bacteria can be cultivated in a suitable medium except in the viable but non-culturable(VBNC) phase (Aladhadh 2023). Stress induces starvation metabolism in many microorganisms. Viable but non Aladhadh-culturable (VBNC) cells cannot grow on suitable media but induce pathogenic pathways (Ramamurthy et al. 2014). These bacteria are hard to detect for food safety (Fakruddin et al. 2013). Different fluorescent dyes are used to detect VBNC bacteria because no colonies will grow. Cell DNA-to-protein ratios determine acridine orange binding to VBNC infections. Reproducing cells are green, while non-reproducing cells are orange. The living cells' enzyme activity is detected by fluorescein isothiocyanate, another VBNC dye. Living cells appear violet or blue (Fakruddin et al. 2013).

Prolonged enrichment and incubation processes limit standard culture methods. Recently, cell wall binding domains (CBDs), which are high-affinity binding molecules derived from bacteriophages, have been utilized as tools for the detection and differentiation of *Listeria* spp. in food. The combination of magnetic separation enhances detection speed and sensitivity, resulting in greater accuracy compared to conventional diagnostic methods. (Schmelcher et al. 2014).

By cultivating extracts from the samples in cell cultures and monitoring the ensuing viral-induced cytopathic effects, viruses in food can be identified. Tissue culture, infectious dose 50 (TCID 50), plaque test, and the most likely number approach are methods for quantifying viruses (Bosch et al. 2011). Numerous studies have used these methods to find viruses in food products that pose serious risks to public health. A number of factors restrict culture-based methods used for viral detection. These factors include the typically low virus content in food, which necessitates the use of extremely sensitive enrichment techniques before detection (Chhabra et al. 2016). This method is unsuitable for rapid testing due to the prolonged duration required to cultivate viruses. Moreover,

several viruses lack cytopathic effects, and effective cell culture techniques are unavailable for some foodborne viruses, including Noroviruses (Bosch et al. 2011; Sánchez et al. 2016). Molecular approaches, such as RT-PCR, are more widely used for viral identification due to their relatively low labor cost, high sensitivity, and accuracy. However, there remain reports of culture-based methodologies being employed to detect of specific viruses (Priyanka et al. 2016).

Ducks, poultry, and wild birds have been discovered to harbor the avian influenza viruses (H7N9 and H5N8), which are a serious public health concern (Dai et al. 2022). According to Shibata et al. (2018), the detection process often comprises homogenizing materials and cultivating aliquots of the homogenate in an embryonated chicken egg (cell culture) for almost -5 days. Hemagglutination is then performed, and a positive result indicates the presence of the virus.

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Microscopic examination for foodborne parasites

The main diagnostic method for the identification of parasites is the direct microscopy technique, which is cheap but time-consuming, needs experience, and is less sensitive (Zolfaghari Emameh et al. 2018).

MALDI-TOF-MS

Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) has recently been implemented in several microbiological laboratories for the routine detection of bacteria and fungi. Despite the substantial initial cost required for a mass spectrometer, the cost associated with detecting a single species is very low when compared with traditional biochemical or molecular genetic methodologies. The application of MALDI-TOF MS reduces the diagnostic process by roughly 24 hours (Hrabák et al. 2013). MALDI-TOF MS is regarded as a crucial element for ensuring food quality and safety (Khater et al. 2021). The use of MALDI-TOF MS to identify food spoilers and food-borne diseases throughout the intricate food chain from farmers and merchants to consumers is growing. Massive food-related microbial isolate samples can be tracked by MALDI-TOF MS because of their dependability, ease of use, cost per sample, and sample throughput. Although their numbers are growing, food spoilers and foodborne pathogen spectra are not well represented in commercial databases. Finding strain-specific biomarkers through proteomics is made possible by tandem mass spectrometry using MALDI-TOF/TOF MS equipment. Multivariate statistics distinguish separate spectra and connect clusters to phenotypes. MALDI-TOF MS molecular identification will become essential for tracking food spoilage and food-borne pathogen strains and isolates (de Koster et al. 2016).

MADLT-TOF MS was utilized to identify Enterobacteriaceae such *as E. coli, Salmonella typhimurium, Klebsiella* spp., *Proteus mirabilis, Enterobacter* spp., *Serratia marcescens, Citrobacter* spp., along with other pathogenic bacteria from minced meat, chicken, fillet fish, fresh milk, yogurt, cottage cheese, and fresh cheese in Egypt (Khater et al. 2021).

MALDI TOF MS has been employed to identify several important fungus groups, including *Rhizopus, Aspergillus, Fusarium,* and *Mucor* (Elbehiry et al. 2017). Using MALDI TOF MS, a range of items from restaurants in the Al-Qassim region of Saudi Arabia have been shown to include foodborne pathogens such *A. niger* and *A. flavus*, as well as other fungal species like *Alternaria alternata*, *P. digitatum*, and *Candida albicans*. Salads, burgers, tortillas, cheese, kabsa, and jareesh are some examples of these dishes (Elbehiry et al. 2017).

MALDI TOF MS has also been used to detect and identify other fungal taxa, such as Mucor (Quéro et al. 2019), and foodborne yeasts (Quintilla et al. 2018) and fungi found in fermented foods (Ahmadsah et al. 2018), have also been detected and identified using MALDI TOF MS." This technology is less commonly used for fungi than for bacteria, mainly because of the methods' adaptation for fungi and the limited availability of a curated fungal spectra database (Bader 2017).

Immunoassays

Immunoassays were developed due to their speed, cost-effectiveness and simplicity. Immunoassays are typically conducted before the utilization of PCR (Leach et al. 2010). The assays encompass serotyping, immunofluorescence, lateral flow devices (LFD), and enzyme-linked immunosorbent assay (ELISA). The principles and mechanisms of action of ELISA and lateral flow devices (LFD), two of the most generally employed immunoassays for the detection of foodborne microorganisms and their toxins, have been extensively investigated (Priyanka et al. 2016; Zhang et al. 2021). The efficacy of immunoassays is contingent upon the purity of antibodies. The specificity of antibodies influences the assay in conjunction with purity. Polyclonal antibodies exhibit reactivity with several epitopes. This may diminish reaction specificity and sensitivity. The reaction matrix contamination may result in the production of false positives (Priyanka et al. 2016).

One of the most generally used immunoassays is ELISA. A significant advantage of utilizing different substrates in ELISA is that they selectively bind to their respective conjugates and result in colorimetric changes that may be measured with a wavelength-based ELISA reader. Even the unaided eye can detect the color change. One drawback is that the chemical and conjugate bond very specifically, and contamination during the intermediary stages could produce false-positive results (Leach et al. 2010; Priyanka et al. 2016).

A. Identification of foodborne bacteria and bacterial toxins by ELISA

ELISA has been employed to identify significant bacterial groupings, including *Salmonella*, *Campylobacter*, *E. coli*, and *Listeria*. Specifically, ELISA has been employed to identify *Salmonella* sp. in meat, dairy products, pasta, and eggs (Wang et al. 2015), as well as *Campylobacter* in food samples (Khan et al. 2018). *Escherichia coli* O157:H7 in cabbage (Pang et al. 2018) and beef (Zhao et al. 2020), *Vibrio parahaemolyticus* in seafood (Kumar et al. 2011) and *Listeria* in milk (Tu et al. 2016). The technique has been employed to identify Staphylococcal enterotoxins (Nagaraj et al. 2016), botulinum toxins in contaminated food (meat and milk) (Stanker et al. 2013), Shiga toxins (Valderrama et al. 2016), and *B. cereus* enterotoxins (Rajkovic et al. 2020) across various food matrices.

B. Identification of foodborne fungi and mycotoxins by ELISA

PCR-ELISA kits have been developed to detect pathogenic *F. verticillioides* and other species. Additionally, it is frequently employed to identify mycotoxins (Hassan et al. 2014). ELISA has been used to identify low amounts of aflatoxin in dried stockfish and aflatoxin B1 from *A. flavus* in preserved corn (Hassan et al. 2014), Low levels of aflatoxin in dried stockfish and aflatoxin B1 from *A. flavus* in preserved corn have both been detected using ELISA (Ounleye et al. 2015), peanuts (Oplatowska-Stachowiak et al. 2016) and soy milk (Beley et al. 2013).

C. Identification of foodborne viruses by ELISA

Immunoassays are generally utilized for point-of-care pathogenic virus detection in clinical and environmental samples, not food only, disease diagnosis, and public health surveillance reasons (Kim et al. 2019). In the past, ELISA immunoassay kits have been used to identify viruses in food products and have been created for the detection of adenoviruses, group A rotaviruses, and astroviruses (Koopmans et al. 2004). However, the advent of nucleic acid-based methods, which provide quicker and more sensitive substitutes, has made them obsolete (Appleton 2000).

The reversed passive latex agglutination assay was more rapid than culture-based methods, as bacterial growth occurred within 6 hours. This was conducted to evaluate the toxigenicity of *Corynebacterium diphtheriae* toxin. Antiserum containing diphtheria toxin was combined with rabbit antitoxin (Toma et al. 1997).

Monoclonal antibodies are superior to polyclonal antibodies due to their monovalent nature, monoclonal antibodies specifically target a single antigen. Its sensitivity and specificity constitute its assets; nonetheless, manufacturing is laborious and costly. Multiple assays have identified *L. monocytogenes*, *S. Typhimurium*, *L. innocua*, and *E. coli.* (Di Padova et al. 1993).

Immunoglobulin G (IgG) initiated a novel approach for targeting pathogenicity in clinical microbiology (Cordeiro et al. 2009). IgY, the chicken egg yolk counterpart of IgG, has progressively gained dominance. The substantial quantities of IgY present in egg yolk and the simplicity of its precipitation purification are its primary advantages. Chicken is appropriate for monoclonal antibodies because of its features. It facilitates immunotherapy and diagnostics (Kovacs-Nolan et al. 2004). This method effectively identified *C. jejuni* at low detection thresholds. A rapid and straightforward gold-labeled immunosorbent assay (GLISA) with a low detection threshold of 7.3 log/cfu/g surpasses several ELISA techniques(Hochel et al. 2004). GLISA is marketed as the Singlepath *Campylobacter* GLISA Rapid Test (Hochel et al. 2004).

Molecular probes for bacterial detection DNAzymes are distinctive. Artificial single-stranded DNA molecules known as DNAzymes are capable of catalyzing chemical reactions. The 'SELEX' process systematic evolution of ligands by exponential enrichment is employed to isolate these compounds from a vast random sequence DNA pool. This method employs a DNA-RNA chimeric substrate at a solitary ribonucleotide junction (R) bordered by a fluorophore (F) and a quencher. The cleavage of the fluorophore and quencher enhances fluorescence intensity, facilitating rapid and uncomplicated bacterial identification (Aguirre et al. 2012). Biochemical and cellular biology research is enhanced by epitope tags possessing distinctive characteristics such as affinity for resins or antibodies, or detection by fluorescence microscopy. *Candida albicans* was identified with this method(Gerami-Nejad et al. 2009).

Nucleic acid- based methods

Molecular biologists employ the sensitive method known as PCR to amplify DNA, PCR is utilized in the detection of parasites, bacteria and viruses (Saravanan et al. 2021), which is considered a sensitive and specific technique in foodborne pathogens diagnosis, there are several types as conventional PCR, Multiplex PCR, real-time PCR, reverse transcriptase PCR, viable PCR and Loop-Mediated Isothermal Amplification (LAMP) (Fig. 2).

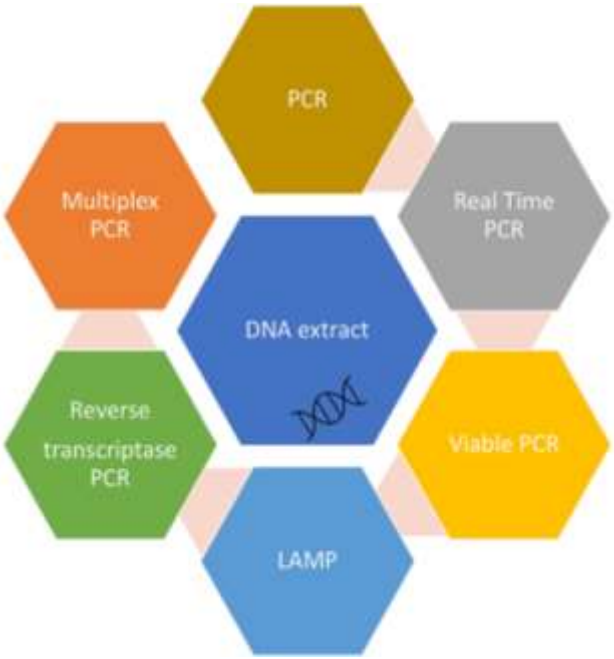

Fig. 2: Nucleic acid-based methods.

The conventional PCR amplifies a specific DNA segment by surrounding a targeted region with a pair of

specific primers. Agarose gel electrophoresis, along with nucleotide sequencing, is necessary for pathogen identification, which can be a time-consuming process (Hill et al. 1996).

These methods have detected *Salmonella* spp., *Campylobacter* sp., *E. coli* O157:H7, *S. aureus*, *B. cereus*, *Enterococcus* spp *L. monocytogenes*, and *V. parahaemolyticus* in ready-to-eat Korean foods (Lee et al. 2014), Minas Frescal cheese (Carvalho 2014), milk (Moezi et al. 2019), beef (Samad et al. 2018), spiked chicken egg, pork, salad (Vinayaka et al. 2019), meat (Algammal et al. 2024) and fish (Pires et al. 2021).

Aflatoxigenic Aspergillus isolates from "meju," a traditional fermented soybean food starter with Korean origins, have been successfully identified using multiplex PCR (Kim et al. 2011). TLC and HPLC studies of filtrates from test cultures confirmed that the specially created primers in this work successfully distinguished between aflatoxin-producing and non-aflatoxin-producing fungi (Kim et al. 2011). *Aspergillus*, *Penicillium*, and *Fusarium* have all been simultaneously identified in contaminated maize grain powder using multiplex PCR (Rahman et al. 2020). PCR can be used in conjunction with cell culture assays, which isolate pathogens via cell culture, or it can be used directly to find viruses in food samples (Bendary et al. 2022). Also, PCR is employed to detect *Clostridium perfringens* toxins in food samples (Bendary et al. 2022).

B-Real-time PCR solved the problem accompanied with low DNA concentration of the pathogens and doesn't need gel electrophoresis. Real-time PCR is considered an accurate quantitative technique, especially for bacterial diagnosis, a more specific and sensitive technique (Fernández et al. 2006). For the molecular diagnosis of parasites, the genomic DNA is isolated from the parasites, the Genomic regions are selected for a PCR-based diagnosis method, and sequencing is required (Zolfaghari Emameh et al. 2018). RT-PCR is used to quantify major foodborne bacterial pathogens in food sample. *Salmonella*, *E. coli*, *Campylobacter* and *L. monocytogenes* were identified and measured by real-time PCR in cheese, chicken, beef burgers, turkey, pig, egg, chicken, and fish (Bai et al. 2022; Chin et al. 2022). A similar method was used to quantify *C. perfringens*, *E. coli* and *S. aureus* in fresh and ready-to-eat vegetables (Azimirad et al. 2021). *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae* have been identified in seafood, shrimp, and mussels using real-time PCR (Azimirad et al. 2021). RT-PCR has been successfully used to identify the Patulin-producing *Penicillium expansum* on intentionally tainted apples (Tannous et al. 2015).

Considering RNA viruses are so common and RT-PCR is so good at identifying foodborne viral infections, it is a commonly used technique (Bosch et al. 2011). When combined with quantitative real-time PCR, which has great sensitivity, specificity, and throughput, this makes it possible to quantify viral particles (Miranda et al. 2019). In Italy, the frequency of Norovirus and Hepatitis A in harvested mussels was investigated. RT-PCR revealed a significant norovirus prevalence, proving beyond a reasonable doubt that eating mussels poses a risk to public health (La Bella et al. 2017). RT-PCR has been employed to detect zoonotic Hepatitis E viruses in uncooked and liver sausages sold in German retail establishments (Szabo et al. 2015) as well as in spiked pork liver sausages (Althof et al. 2019) and in raw and liver sausages available in retail outlets in Germany (Szabo et al. 2015). This Brazilian study assessed the levels of rotavirus, hepatitis E virus, and adenovirus in market-sourced beef, hog, and chicken meat pieces. The results illustrated that, of the samples examined, Rotavirus was the most significant viral pathogen found in chicken chops (Soares et al. 2022).

. In comparison to Real-Time PCR and traditional PCR, Transcriptase C-Reverse is utilized less often for the detection of foodborne bacterial infections.

This happens because mRNA is rapidly broken down in the food matrix, resulting in false negative results even though it is a more accurate predictor of bacterial vitality (Xiao et al. 2012). In comparison to other approaches, this methodology is less commonly used for detecting foodborne germs since it is labor-intensive (Foddai et al. 2020).

Bacterial pathogens in a variety of food samples have been identified using this technique, such as *S. Typhimurium* in infected jalapeños and serrano peppers (Miller et al. 2010) and *S. enterica* in milk and spiked egg broth (Zhou et al. 2014). D-Viable PCR (vPCR), a PCR variation used for sample pre-treatment, uses intercalating dyes such as ethidium monoazide (EMA) and propidium monoazide (PMA) followed by PCR amplification. This method provided for the detection of living foodborne pathogens (Chen et al. 2022), vPCR may offer a more accessible approach for identifying pathogens compared to RT PCR. By creating permanent links with the DNA molecules of weakened or nonviable bacterial cells, the intercalating dyes can penetrate their membranes and prevent PCR primer amplification. Any amplicon found in the subsequent PCR will, therefore come from living bacterial cells (Chen et al. 2022). This technique has been employed to detect live *Salmonella* sp. in spiked pork meat and ready-to-eat salad (Dinh Thanh et al. 2017) as well as *Helicobacter pylori* in retail pork meat (De Cooman et al. 2013), highlighting the effectiveness of this approach in evaluating food safety (Dinh Thanh et al. 2017). The integration of vPCR with Real-Time PCR has facilitated the precise detection and quantification of viable *Campylobacter* sp. in frozen and chilled poultry meat (Castro et al. 2018) and *Listeria* sp. in spiked meat, salad, cheese, and milk (Agustí et al. 2018).

E-LAMP has been utilized for the detection of *V. vulnificus* and *V. parahaemolyticus* (Cao et al. 2019) in food items, particularly seafood LAMP assays have been established for the rapid identification of ochratoxin-producing *Penicillium nordicum* in meat products which is dry-cured (Ferrara et al. 2015).

Next-Generation Sequencing (NGS) methods

The capabilities of NGS and bioinformatics have significantly enhanced food safety. NGS is mostly employed in the sequencing of whole genomes and metagenomics to ascertain the genomes of various microorganisms within a sample. The application focuses on bacteria, fungi, and viruses by employing 16S rRNA, ITS, or alternative biological markers (Jagadeesan et al. 2019). NGS has multiple food safety applications.

NGS has been employed to validate the foodborne bacterial pathogens detection. NGS may identify pathogens

such as *Salmonella* in fresh food (Lewis et al. 2020). Various NGS techniques (16S amplicon sequencing, RNA-seq employing ScriptSeq, and NEBNext kits from New England BioLabs) were utilized to evaluate the limit of detection (LoD) for *Salmonella* and MS2, a surrogate for Norovirus. The ScriptSeq approach exhibited the highest sensitivity, demonstrating a Salmonella limit of detection (LoD) of $10^{\wedge 4}$ CFU per reaction and a phage MS2 LoD of $10^{\wedge 5}$ PFU per reaction (Lewis et al. 2020). A literature analysis revealed that whole genome sequencing (WGS) has detected *Listeria monocytogenes* strains in foodborne disease outbreaks (Law et al. 2015). Shiga toxin-producing *E. coli* was detected in spinach spikes by shotgun metagenomics (LoD ~10 CFU/100 g) (Leonard et al. 2015).

Multiplex PCR-based NGS (Illumina sequencing) has identified *S. sonnei*, *L. monocytogenes*, *C. jejuni*, *S. enterica* subsp. enterica serovar enteritidis, *S. aureus*, *E. coli*, and *Y. enterocolitica* in meat (pork and poultry), milk, shellfish, and vegetables (Ferrario et al. 2017). Next-generation sequencing (amplicon-based Illumina sequencing) has identified *Aeromonas hydrophila* and *Rahnella aquatilis* in ready-to-eat salad (Mira Miralles et al. 2019). Whole genome sequencing via next-generation sequencing (NGS) is advantageous for the surveillance of foodborne pathogens (Moran-Gilad 2017). Fungal pathogens are subjected to less next-generation sequencing compared to bacterial pathogens. It has been employed to identify pathogens in clinical and environmental specimens (Jiang et al. 2022).

RT-PCR is employed to identify positive samples before the confirmation using NGS sequencing. The sequencing of whole genome was utilized for phylogenetic analysis of the Avian influenza virus from the poultry meat products of international visitors and samples from the Tongzhou poultry meat market in China (Cui et al. 2022). In 2012, RT qPCR, dPCR, and NGS (metagenomics) were employed to identify and classify the Norovirus genotype in frozen strawberries in Germany (Bartsch et al. 2018). Next Generation Sequencing (metagenomics) was employed to identify Hepatitis A and/or Norovirus viruses in RT-PCR-positive samples from celery (Yang et al. 2017), oysters sourced from Japanese aquaculture (Imamura et al. 2017), and RT qPCR-positive lettuce, parsley, and strawberry samples from Spanish organic farms (Itarte et al. 2021). The amplicon-based next-generation sequencing approach has been employed to identify and classify foodborne viruses. Nested PCR aimed at NoV and HPV genes, utilizing Illumina adapters in the primers, revealed considerable diversity of Hepatitis A viruses and Noroviruses in RT qPCR-positive samples of organically cultivated fresh lettuce, parsley, and strawberries (Itarte et al. 2021).

Biosensor

Biosensors represent the most recent advancements in detection systems, with certain models exhibiting superior detection limits that substantially mitigate or remove the limitations inherent in PCR procedures. Biosensors are utilized for the detection of pathogens that typically comprise three components: a biological capture molecule (such as probes and antibodies), a mechanism for transducing the interactions between the capture molecule and the target into a signal, and an output data system (Chattaway et al. 2011). Notwithstanding improved detection efficiency, outcomes obtained by molecular biology techniques may be influenced by diverse dietary matrices. The primary benefit of biosensors is their ability to identify infections at low detection thresholds with high specificity and sensitivity; yet, they necessitate highly specialized and costly devices, together with suitable software, to yield precise findings. Consequently, these solutions may not consistently be cost-effective (Chattaway et al. 2011; Priyanka et al. 2016).

Electrochemiluminescent assays are performed on 96-well plates, and they rely on the electrochemical excitation of reporters such as ruthenium (IItris-bipyridyl (Ru(bpy)3)2+ chloride. This method can be used to detect low concentrations. An improved version of this is called the cytometric bead assay, implemented using a fluidic technique and employing red and infrared fluorophores. Upon application to electrode 1, this results in orange fluorescence. A lab-on-a-chip includes cell pre-concentration, purification, PCR and capillary electrophoretic analysis. It consists of a 100 nl-PCR reactor and a 5cm long CE column for amplicon separation. The detection limit is 0.2 CFU/μL (Zhu et al. 2012). It can be employed for the detection of *E. coli* K12. Analogous to lab-on-a-chip assays, a cell phone-based platform for *E. coli* detection facilitates the liquid samples screening. Inexpensive, battery-operated light-emitting diodes are utilized. The sample is agitated, and the emission from the quantum dots is captured using a smartphone camera. Detection of *Salmonella* sp. in fat-free milk was achieved with a limit of 5 to 10 CFU/mL. Likewise, antibody-quantum dot conjugates were employed to identify *E. coli* and *S. Typhimurium* (Dwarakanath et al. 2007). CdSe/ZnS quantum dots demonstrated a shift in fluorescence emission when coupled to antibodies or DNA aptamers attached to bacteria. The alteration in emission peak transpires when the quantum dots interact with the bacterial surface (Dwarakanath et al. 2004).

A surface plasmon resonance (SPR) was developed via a subtractive inhibitory technique with goat polyclonal antibodies targeting *E. coli* O157:H7. The findings indicated that the signal had an inverse correlation with the concentration of *E. coli* O157:H7 (Wang et al. 2011). Fluorescence resonance energy transfer (FRET) employs an antibody that identifies cell surface epitopes of the target cell. It utilizes complementary oligonucleotides modified with fluorochromes. The fluorescence is identified through the utilization of sensors. FRET was employed to detect *E. coli* O157:H7 and Salmonella. The advantage of FRET is its simplicity and rapidity, yielding results within five minutes. It is cost-effective and exceptionally sensitive (Heyduk et al. 2010).

Optical biosensors have demonstrated superior detection systems and pathogen separation capabilities. These biosensors comprise optical fibers, planar waveguides, surface plasmon resonance (SPR) and microarrays. Their compact design and label-free detection facilitate specific and sensitive identification, constituting a significant benefit of optical biosensors (Narsaiah et al. 2012).

Nanobiotechnology

Nanobiotechnology represents the most recent

methodology for infection identification. The development of sensors for proteins, DNA, and small molecules is attracting increasing interest in aptamers. In contrast to the traditional culture, an experimental design that combined polydiacetylene and nucleic acid aptamer showed a 98.5% detection rate of *E. coli* O157:H7 in 203 clinical fecal samples (Priyanka et al. 2016). High affinity and specificity are characteristics of aptamers (Wang et al. 2008). Aptamers have made use of fluorescence-displaying silver nanoparticles, bioconjugated nanoparticles, and gold nanoparticles (GNPs). GNPs have unique applications due to their electrical, photonic, and catalytic properties (Wang et al. 2008). Because of their optical properties, GNPs can be used in colorimetric techniques. These compounds can easily conjugate to antibodies and are non-toxic. A bioconjugated, nanoparticle-based bioassay generates a robust fluorescent signal for bioanalysis (Zharov et al. 2006). An experiment was conducted utilizing *E. coli* O157 cells in a beef sample within a 384-well microplate configuration. A novel method entails inflicting physical harm on bacteria by the utilization of pulsed laser radiation in conjunction with absorbent nanoparticles. Upon irradiation, nanoparticles absorb energy and subsequently release heat during relaxation, resulting in cellular damage. GNPs have been utilized for this strategy (Zharov et al. 2006).

DNA microarray

Because of its speed, sensitivity, and specificity, which enable high-throughput research, DNA microarray is becoming a more important and useful tool. Numerous studies have been conducted to discover marine fish infections and waterborne diseases that indirectly endanger humans through fish consumption. Foodborne pathogen microarrays that are particularly made to target internal transcribed spacer (ITS) sequences were identified by (Wang et al. 2007). Ten pathogens' existence in PIF was investigated in a study (Wang et al. 2009). In addition to *E. sakazakii, C. freundii, K. pneumoniae, K. oxytoca, Serratia marcescens, and A. baumannii,* this method identified *B. cereus, E. coli, L. monocytogenes, P. aeruginosa, S. enterica, S. aureus*, and *V. parahaemolyticus* as being associated with PIF6 contamination. These infections are identified using the DNA microarray technique. Because of the high DNA homology that makes distinction difficult, the ITS regions of five Bacillus species (*B. anthracis, B. cereus, B. thuringiensis, B. mycoides, and B. weihenstephanensis*) were examined. This problem was resolved by the DNA microarray (Wang et al. 2009). PulseNet, a national genetic subtyping network for foodborne disease surveillance, is important in identifying each outbreak caused by microorganisms. It primarily aids in diminishing product recalls, restaurant closures, and associated processes following the outbreak. This is conducted in municipal, state, and public health regulatory agency laboratories (Boxrud et al. 2010).

Conclusion and prospects for the future

Foodborne pathogens (bacteria, viruses, fungi, or parasites) continue to pose a significant risk for public health, food safety, and worldwide economies. Although organisms such as Salmonella, *E. coli* and Listeria are well-known offenders, other pathogens, such as noroviruses, hepatitis viruses and parasites like Cryptosporidium and Toxoplasma, are also significant causes of foodborne illness. However, other pathogens remain, developing strategies to withstand these stress environments, therefore making detection increasingly complex. Culture-based techniques that rely on pre-existing knowledge of the pathogen are slow, which necessitated the development of faster molecular diagnostics based on approaches like polymerase chain reaction (PCR), next-generation sequencing (NGS), MALDI-TOF-MS, ELISA, Biosensor, Nanobiotechnology and DNA microarray. However, even with advancements, parasitic infections are still largely neglected, in part due to the chronic nature of these diseases as well as their presence in areas with high levels of poverty and sanitation compromise. Novel diagnostics and global surveillance are needed for these neglected pathogens and to address the threat they pose to global health systems during microbial and viral pandemics.

Prospects for the future

- Improving rapid detection through Creating affordable, portable biosensors and point-of-care tools for monitoring pathogens in food and water supplies in real time.
- Integrating omics technologies by increasing the application of proteomics, transcriptomics, and genomics to gain a deeper understanding of pathogen behavior, resistance mechanisms, and transmission patterns.
- In order to reduce zoonotic foodborne pathogens that spread from animals to humans, the one health approach recognizes how closely human, animal, and environmental health are connected.
- Promoting stronger laws governing food handling and pathogen control while educating communities about food safety procedures is known as public awareness and policy.

When we combine the latest scientific advances with public health strategies, we can lessen the burden of foodborne illnesses and build a safer and healthier future for all.

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