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FOOD UNDER THE MICROSCOPE: NEW FRONTIERS IN PATHOGEN DETECTION

[1]Vinay Naik, [2]Dr. Nitinkumar Patil, [3]Rupali Devarkonda

[1]Ph.D. Student, [2]Assistant Professor, [3]M.Sc. Student

[1]Department of Microbiology,

[1]Smt Chandibai Himathmal Mansukhani College Ulhasnagar, Dist. Thane, India

Abstract

Foodborne pathogens remain a critical public health challenge worldwide, responsible for severe illness, hospitalizations, and economic losses. Key bacterial culprits such as *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus* are frequently linked to contaminated food and water sources. While culture-based microbiological methods have long been used for pathogen identification, they are often time-consuming and lack the speed required for rapid response. This review highlights recent advancements in molecular and immunological detection methods that have revolutionized food safety monitoring. Molecular approaches—including polymerase chain reaction (PCR), real-time PCR (qPCR), multiplex PCR, loop-mediated isothermal amplification (LAMP), and reverse transcription PCR (RT-PCR)—offer rapid, sensitive, and specific identification of pathogens even at low concentrations. Immunological methods, particularly enzyme-linked immunosorbent assay (ELISA) and lateral flow assays, are valuable for their ease of use, cost-effectiveness, and potential for on-site testing. While these methods are powerful, each has limitations such as cross-reactivity, inability to differentiate viable from non-viable organisms, or dependence on specialized equipment. The integration of these approaches with biosensors, isothermal systems, and portable platforms shows promise for enhancing real-time detection capabilities. Ultimately, a combined strategy leveraging both traditional and next-generation methods is essential to prevent foodborne outbreaks and protect consumer health. This paper underscores the importance of ongoing innovation and interdisciplinary collaboration in ensuring food safety.

Index Terms / Keywords:

Foodborne pathogens; PCR; ELISA; molecular diagnostics; food safety; pathogen detection.

I. Introduction

Microorganisms, predominantly bacteria, are naturally present in the human body as normal flora, specifically on the skin and gastrointestinal (GI) tract. These microorganisms generally benefit, aiding in various essential bodily functions. However, pathogenic microorganisms, including bacteria, viruses, and fungi, pose a significant health risk when they enter the body. One of the major routes through which these pathogens enter is the gastrointestinal tract, leading to numerous foodborne diseases. Pathogens can enter the body via contaminated food, beverages, or water, or through the consumption of undercooked food. Thus, detecting pathogens in food and water sources before consumption is crucial for preventing serious health outbreaks and ensuring public safety. These pathogens include, but are not limited to, *Acinetobacter spp.*,

Bacillus subtilis, *B. cereus*, *Campylobacter jejuni*, *Citrobacter koseri*, *C. freundii*, *Clostridium difficile*, *C. perfringens*, *Enterobacter sakazakii*, *E. cloacae*, *Escherichia coli O157:H7*, *Klebsiella oxytoca*, *K. pneumoniae*, *Listeria monocytogenes*, *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Shigella sonnei*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Yersinia pestis* (1–8). The detection of these pathogens is of paramount importance in several sectors, including public health, water and food industries, pharmaceuticals, environmental monitoring, and biodefense (7, 9).

Among the various foodborne pathogens, *Shiga toxin-producing Escherichia coli* (STEC), particularly *E. coli* O157:H7, has been implicated in several severe foodborne outbreaks. This pathogen is categorized as enterohemorrhagic *E. coli* (EHEC), possessing characteristics of both verotoxigenic *E. coli* and a lesser-known type of diarrheagenic *enteraggregative E. coli*. Contaminated water, including that found in swimming pools, has also been identified as a source of *E. coli* O157:H7 infection, as observed in Mangalore, Karnataka, India (10).

The first reported outbreak of *E. coli* O157:H7 in China occurred in 1986 (11), with subsequent cases emerging across the country in regions such as Fujian, Gansu, Zhejiang, Jiangsu, and Anhui (11, 12). Powdered infant food (PIF), particularly powdered milk, has been a major concern regarding bacterial contamination, with outbreaks of *E. sakazakii* and *Salmonella* traced back to such products. For example, in 2002, powdered milk produced by Wyeth was found to be contaminated with *E. sakazakii* (7). In France, PIF was similarly contaminated with *Salmonella spp*. (6), and in Trinidad, a study of 15 farms found the presence of *Salmonella* (13). Germany experienced one of its worst outbreaks of *E. coli* O157:H7 in 2011, which resulted in an unusually high number of Hemolytic Uremic Syndrome (HUS) cases (2). Furthermore, countries like Turkey have also reported large-scale outbreaks attributed to beef consumption, with a significant population consuming contaminated meat (14).

Several other regions across the world, including Mexico, Ireland, Belgium, England, France, and Poland, have reported the presence of *E. coli* O157:H7 in cattle farms, carcasses, and feces (14). Additionally, Canada has reported STEC detections in stool samples screened for viral gastroenteritis (13). In Tanga, Tanzania, a region central to milk production, various pathogens have been detected in milk, highlighting the importance of proper pathogen control in dairy products (15). Similarly, mozzarella cheese, a widely consumed product, has also been found to be susceptible to contamination by *Listeria monocytogenes, E. coli, and Pseudomonas fluorescens* in Oregon, USA (16). Fermented milk-based beverages, such as kefir, which have low alcohol content, are also prone to bacterial contamination (4). One of the most concerning pathogens, *Listeria monocytogenes*, can grow at temperatures as low as 4°C, which is especially problematic for refrigerated foods (17). The ability of *Listeria* to thrive at low temperatures necessitates timely testing to prevent fatal outbreaks, particularly for immune-compromised individuals, pregnant women, and the elderly.

In addition to *Listeria*, *Staphylococcus aureus*, including methicillin-resistant strains (*MRSA*), has also been a significant concern in foodborne outbreaks in countries such as China (18) and Spain (19). *S. aureus* is notorious for producing toxins that are heat-stable, making it a challenge to prevent in foods that undergo minimal cooking or handling.

The global impact of foodborne pathogens underscores the need for effective detection methods. Early identification and control of these pathogens are essential in preventing large-scale outbreaks, particularly among vulnerable populations such as infants, the elderly, and immune-compromised individuals. The rapid detection of foodborne pathogens has been a focal point of research, with several methods developed and refined over the years. These methods are categorized based on principles such as immunological detection, molecular techniques, and microbiological culture, each offering unique advantages and limitations. This review provides an overview of these detection methods, discussing their principles, advantages, disadvantages, and applications in various industries. The methods range from traditional microbiological techniques, such as culturing and biochemical tests, to more modern approaches like PCR (polymerase chain reaction), enzyme-linked immunosorbent assay (ELISA), and biosensors.

For instance, PCR-based techniques have revolutionized the rapid detection of foodborne pathogens, allowing for the identification of pathogens even at low concentrations. Similarly, immunological methods like ELISA have enabled the detection of specific antigens or antibodies, offering a rapid and cost-effective alternative to traditional microbiological methods. However, each method comes with its own set of

challenges, such as the need for specialized equipment, the potential for false positives or negatives, and the time required for results. (20)

II. Methodology

This review was conducted by analysing peer-reviewed articles, scientific reports, and case studies related to the detection of foodborne pathogens. Literature was sourced from databases including PubMed, Science Direct, Google Scholar, and Web of Science, focusing on publications from 2000 to 2024. Keywords used included: foodborne pathogens, PCR detection, ELISA, molecular diagnostics, rapid detection methods, and names of specific pathogens such as E. coli O157:H7, Salmonella spp., and Listeria monocytogenes.

Articles were selected based on relevance, scientific rigor, and their focus on detection methods in food or water matrices. Both culture-dependent and culture-independent approaches were reviewed, with emphasis on molecular (e.g., PCR, LAMP, RT-PCR) and immunological techniques (e.g., ELISA, lateral flow assays). Studies comparing detection performance—sensitivity, specificity, speed, and practicality—were prioritized to highlight current innovations and limitations in food safety diagnostics.

III. Results

Understanding Major Foodborne Bacterial Pathogens

Hippocrates (460 B.C.) was the first to highlight the correlation between food consumption and human illness, recognizing the impact of foodborne diseases. Foodborne pathogens, including bacteria, viruses, and parasites, are responsible for outbreaks, defined as the occurrence of two or more similar illness cases caused by the consumption of a common food (21). Microorganisms, primarily bacteria, are typically harmless and beneficial in the human body, contributing to essential functions (22). However, harmful pathogens, such as bacteria, fungi, and viruses, can cause illnesses, especially when they enter through the gastrointestinal tract via contaminated food or water (23). Recognizing and detecting these pathogens in food and water is vital to prevent outbreaks (24). Common foodborne pathogens include *Acinetobacter spp.*, *Bacillus subtilis*, *E. coli O157:H7*, *Listeria monocytogenes*, *Salmonella*, and *Staphylococcus aureus* (25, 26, 27).

1. ***Escherichia coli (E. coli)***

E. coli is a Gram-negative, rod-shaped bacterium, some strains of which are harmful due to toxin production (28, 29). Pathogenic strains like *E. coli* O157:H7 cause severe illness, with complications such as Hemolytic Uremic Syndrome (HUS). Contamination occurs via food or water contaminated by fecal matter (29). Pathogenic *E. coli* are classified into six groups based on pathogenic mechanisms: EPEC, EHEC, ETEC, EIEC, and A/EEC (29, 30). *E. coli* O157:H7 is a major foodborne pathogen, responsible for severe outbreaks, especially in fresh produce (31).

2. ***Bacillus cereus***

Bacillus cereus is a Gram-positive, motile bacterium capable of producing spores. It is commonly found in food production environments and can contaminate a wide range of foods. The bacterium produces virulence factors that cause gastroenteritis, typically mild but transient in nature (32). *B. cereus* spores can adhere to surfaces, making contamination difficult to prevent, particularly in rice dishes, which are often associated with outbreaks (33).

3. ***Salmonella spp***

Salmonella are Gram-negative, facultative anaerobes known for causing gastroenteritis, among other infections. The genus comprises two main species: *Salmonella enterica* and *Salmonella bongori*, with several subspecies that infect both humans and animals (34). Salmonella infections are a major public health concern, with *Salmonella Enteritidis* and *Salmonella Typhimurium* being the most commonly identified in foodborne outbreaks. Infection typically results from consuming food contaminated by infected animal products or fecal matter (31). *Salmonella* is a leading cause

of foodborne illness globally, with significant morbidity and mortality, especially in developing nations (35).

They infect humans and animals with a wide range of infectious diseases. The most prevalent of these illnesses is gastroenteritis, which is characterised by diarrhoea brought on by the inflammatory response and possibly by toxins as well as bacterial growth in the intestinal submucosa (36). Four clinical kinds of *Salmonella* infections have been identified: convalescent lifetime carrier condition, enteric fever, bacteremia or septicaemia, and gastroenteritis.

The most common bacterial cause of food-borne disease in the United States is *Salmonella spp*. (31). According to CDC estimates, about 1 million Americans get *Salmonella* annually, resulting in an average of 19,000 hospitalisations and 380 fatalities (31). Many wild animals and the majority of livestock have intestines that are home to *Salmonella spp*. Infection with *Salmonella spp*. typically happens when a person consumes food tainted by the excrement of animals or people who are infected. Although *Salmonella* outbreaks are frequently linked to meat, poultry, and eggs, other foods including fruits and vegetables can also become contaminated by these bacteria. More recently, the CDC announced that 258 people from 24 states and the District of Columbia had contracted the outbreak strain of *Salmonella bareilly* (247 person), and *Salmonella Nchanga* (11 person) (37). No fatalities have been reported, and thirty-two sick people have been admitted to hospitals. The probable cause of this outbreak is a frozen raw yellow fin tuna product called Nakaochi Scrape from Moon Marine USA Corporation, according to cooperative investigation efforts by state, local, and federal public health officials (37).

In conclusion, the detection of foodborne pathogens like *E. coli*, *B. cereus*, and *Salmonella* is critical to preventing outbreaks and safeguarding public health. Effective detection methods are essential for identifying contamination early and minimizing the impact of foodborne diseases.

Various Methods for detecting food borne pathogen:

Pathogenic bacteria in food can be found using a variety of methods, which fall into two categories: biological and biochemical detection methods. Enzyme-linked immunosorbent assay (ELISA), enzyme-linked fluorescent assay (ELFA), lateral flow assay, and magnetic separation techniques are all part of the immunological-based biochemical detection method (38). In contrast, biological methods can be classified as either culture-dependent or culture-independent.

1. Biochemical methods:

The biochemical method gives microbiologists some clues to identification by using a variety of conventional methods to detect pathogens that were grown on agar plates. Various chemical properties are essential to the detection procedure. There are many different biochemical assays that can be used to identify the infections (38). Chemical properties like the synthesis of indole, citrate utilisation, catalase, oxidase, starch hydrolysis, carbohydrate fermentation, methyl red, triple sugar iron agar, Voges-Proskauer, and nitrate reduction tests have all been employed. In order to identify microorganisms. Nucleic acid-based and immunological-based techniques were developed and are currently being used to provide greater accuracy and specificity. (39)

2. Biological methods

2.1 Culture dependent methods:

Culture-dependent approaches take longer and are more time-consuming. The procedure gets overly involved when food items (pathogens impacted) are sampled, serially diluted, inoculated on media that are suitable and selective for bacterial growth, and then incubated. The incubation period for food-borne diseases can range from 12 to 24 hours, depending on the bacterial strains, to up to 72 hours. Finding out how many bacteria are still alive during the growth and death rate measurements becomes essential as well. The colony-forming units on the sample show the bacterial gradation (40).

Using the enzymes' ability to produce light during enzymatic processes, bioluminescence is a traditional technique for detecting pathogens. For a long time, phage typing has been employed as a biological tool for pathogen detection. This technique is typically employed to find a specific bacterial strain and identify the

source of the infection in the sample (41). Bacteriophage is utilised in biochemical assays instead of antibodies, making it an effective biological tool (42).

2.2 Culture independent methods:

The primary goal of food microbiologists is the rapid and accurate identification and detection of food pathogens (43). The advent of molecular-based techniques made it simpler for microbiologists to achieve their objectives. Molecular-based methods such as PCR and ELISA are known for their quick and multiple detection (44). Following this, the field expanded significantly, opening the door for novel detection methods including electrospray ionization–mass spectroscopy (ESI–MS) and matrix-assisted laser desorption/ionization–time of fight (commonly known as MALDI–TOF) (45). These methods aid in the identification of nonviable microbial cells in addition to producing reliable data (46).

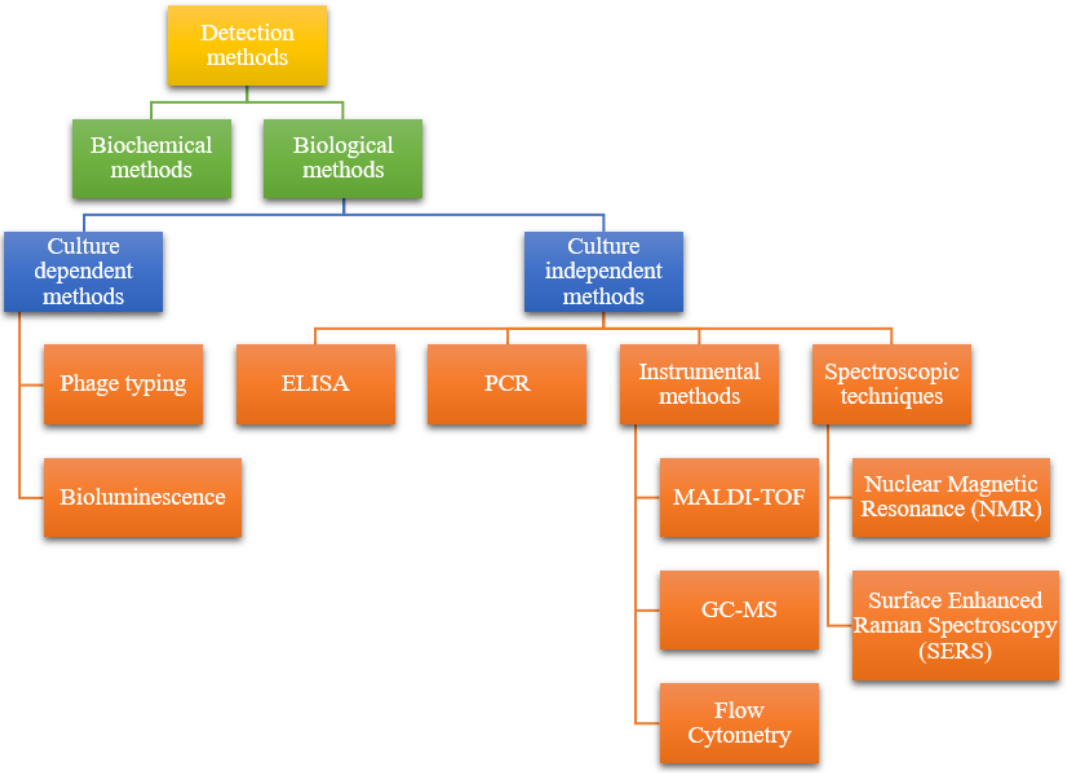

Figure 1. Overview of modern detection methods for foodborne pathogens, categorized into biochemical and biological approaches. Biological methods are further classified into culture-dependent and culture-independent techniques, highlighting key technologies including immunoassays, PCR, and advanced instrumental and spectroscopic methods.

3. Immunoassays:

Immunoassays evolved because they were less costly, easier to use, and produced results more quickly. Therefore, immunoassays are typically carried out prior to Polymerase Chain Reaction (PCR) based techniques. One of the most popular immunoassays available today is Enzyme Linked Immunosorbent Assay (ELISA). The success of immunoassays is significantly influenced by antibody purity. The specificity of the antibody is another element that influences the assay in addition to purity. Polyvalency, or having several epitopes to react with, is a feature of polyclonal antibodies. Low specificity and sensitivity may result from this impact on the reaction. It should be mentioned that false positive results are possible. One such finding was a cross-reaction between *Brucella abortus*, *Y. enterocolitica* O:9, and *E. coli* O157:H7, all of which were isolated from serum samples of calves that were afflicted (35).

One significant benefit of using different substrates in ELISA is that they will bind to the appropriate conjugates selectively and produce colouration that can be detected by an ELISA reader using wavelength. The colour shift is apparent to the unaided eye. One drawback, though, is that the chemical and conjugate

attach very specifically, and contamination in the middle phases can result in a false-positive test. Bovine serum albumin (BSA) solution29 interacts with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), which is one such substrate that is produced in 0.05 M phosphate-citrate buffer. Another substrate that is most frequently used in ELISA is tetramethylbenzidine. It attaches itself to HRP, or horse radish peroxidase.

The colouration takes time to develop. Sandwich ELISA was developed using this technique to detect *Listeria sp* (47). P-nitrophenyl phosphate (pNPP), another frequently utilised substrate, binds only to BSA linked alkaline phosphatase (48). pNPP was employed as the substrate in one of the studies that detected *E. coli* O157:H7 (49). Bispecific antibodies were created to identify the foodborne pathogen *L. monocytogenes* and human red blood cells (RBCs). This is based on the idea that a mixture of anti-RBC and anti-Listeria antibodies is first reduced, and then the reduced disulphides are gradually reoxidised. This makes it easier for the divided antibody chains to bind together and create hybrid immunoglobulins that have a higher affinity for human red blood cells and *L. monocytogenes*. Only when *L. monocytogenes* cells were present did the bispecific antibodies cause the RBCs to clump together. When *L. monocytogenes* was present, the agglutination process produced red clumps that were easily apparent to the unaided eye. It was discovered that this was a straightforward method for quickly and extremely precisely screening different diseases in their biological habitats (50).

ELISA techniques have occasionally been enhanced to accommodate the constantly developing novel investigations. *E. Coli* O157:H7 LPS was used as the antigen in the construction of the blocking ELISA. These proved to be more sensitive than the standard ELISA (38) and were successful in identifying the infection in cattle. Anti-O157 antibodies have been found in both human and animal serum using indirect ELISA. Cross-reactivity, however, increased the likelihood that the result would be a false positive (51).

A modified version of ELISA called sandwich ELISA uses two antibodies to detect a single antigen. Compared to the current tests, the sensitivity and specificity are significantly greater. Shiga-like toxin (stx) in E. coli O157 strains, non-O157 STEC strains, and Listeria sp (4) was found to be detectable using this type of ELISA. Here, HRP was utilised as a conjugate for the detection of a polyclonal antibody. The Sandwich ELISA, also referred to as the SEF 14 double-antibody sandwich (DAS) ELISA, has been optimized to enhance the detection of antibodies specific to the SEF 14 fimbrial antigen. This is used to identify flocks of chickens that have *S. enteritidis* infections (52). It could distinguish between chickens infected with *Salmonella panama and S. Typhimurium* and those infected with *S. enteritidis*. In a separate sandwich ELISA assay, the Shiga toxin 2a (stx2a) antigen was successfully detected in spiked soil and fecal samples, with detection limits ranging from 10–100 pg/mL for soil and 100–500 pg/mL for fecal material. When the same samples were evaluated using PCR, the method exhibited 100% sensitivity and specificity, confirming its high accuracy and reliability in pathogen detection (24).

The primary benefit of the reversed passive latex agglutination assay was that the bacteria could grow in just 6 hours, which led to a faster result than the culture-based method (53). This approach was employed to determine the toxigenicity of *Corynebacterium diphtheriae* by detecting the presence of diphtheria toxin (53). The method involved reacting diphtheria toxin with a rabbit-derived diphtheria antitoxin antiserum, enabling the identification of toxin-producing strains through immunological interaction.

Due to their monovalency, monoclonal antibodies are favoured over polyclonal ones. The production of monoclonal antibodies targets a single antigen. Although its main advantages are sensitivity and specificity, manufacture is time-consuming and not economical. Numerous such tests have been carried out to identify *E. coli, S. Typhimurium, L. innocua, and L. monocytogenes* (54).

4. PCR based methods:

Kary Mullis discovered PCR in 1985, which is regarded as one of the seminal discoveries in recombinant DNA technology (55). The idea behind PCR is that genes of different pathogens can be amplified and further studied (56, 57). Each target gene is amplified using specific primers, and the resulting PCR products are subsequently analysed through agarose gel electrophoresis. Following electrophoresis, the DNA fragments are visualized by staining with ethidium bromide, which intercalates into the DNA and fluoresces under UV light, allowing for the detection and size estimation of the amplified products.

Since the discovery, different types of PCR have evolved, each with its own unique protocol. Generally speaking, the main benefits of PCR are that it is a quick and sensitive procedure that is quicker than culture-based methods and immunoassays. By using several primer pairs, it is now much easier to identify between strains, and PCR has advanced to the point where the amplified product can be acquired in about 30 minutes. The detection limit for DNA amplicons in PCR-based assays can reach as low as femtogram levels (10^{-15} g), demonstrating the high sensitivity of this method. Advances in molecular techniques and optimization of reaction conditions have contributed to progressively improving these detection thresholds over time (29).

In food safety labs, this may serve as an alternative to the laborious and time-consuming process of growing and identifying pathogens (57). Although low detection limit will continue to be the primary criterion, the method has not remained cost-effective due to advancements in PCR technology. The PCR approach has emerged as a very promising way to identify the genes in pathogens, but it has some drawbacks that necessitate the development of more effective techniques. Cell lysis and nucleic acid extraction are among the challenges, as are cross-contamination and unsuccessful reactions brought on by the presence of inhibitory substances or competing DNA from non-target cells. Due to the possibility of inconsistent results, PCR may become less appealing as a dependable method (22). Live and dead cells cannot be distinguished using PCR techniques. The possibility of producing a false-positive result because of binding to non-specific double-stranded DNA sequences is the main drawback of all PCR techniques. Therefore, it is critically important to design highly specific primers that do not amplify non-target sequences, as non-specific amplification can lead to false-positive results and compromise the accuracy and reliability of the PCR assay.

The development of PCR-based methods marked one of the earliest and most transformative advancements in molecular cloning and recombinant DNA technology, significantly enhancing the speed, sensitivity, and specificity of foodborne pathogen identification.

One of the techniques involved designing appropriate primers for amplification based on the *Salmonella* gene *fimA* and the pathogenic *E. coli* gene *afa*. When compared to marker DNA, the amplified product's size was 120 bp. This method for detecting *Salmonella* and harmful *E. coli* is quick, accurate, and sensitive (58). In loop-mediated isothermal amplification (LAMP) assays, the target genes selected included *stx1*, *stx2*, and *eae*—all key virulence markers in Shiga toxin-producing *Escherichia coli* (58). LAMP enables rapid amplification of up to 10^9 copies of target DNA within an hour under constant temperature conditions (60–65°C), utilizing four to six specially designed primers and a strand-displacing Bst DNA polymerase. This method offers a simple, efficient, and highly sensitive alternative to traditional PCR techniques. The performance of LAMP was compared with that of quantitative PCR (qPCR), demonstrating comparable accuracy while offering faster turnaround and reduced equipment needs.

Within one hour, the outcome was achieved. This approach was proven to be quick, specific and sensitive for the detection of STEC strains. The lack of false-positive or false-negative results is an additional benefit (58). Early detection of the pathogen's presence is crucial during every outbreak. When paired with a rapid cycling apparatus, real-time PCR enables target quantification and produces data within 30 minutes of the heat cycling beginning. For the identification and measurement of microorganisms, real-time qPCR is regarded as the preferred technique. Its speed advantage over CC-based techniques is one of its main benefits. Additionally, it allows for the simultaneous identification of many microbes and is very sensitive and specific (59). A modified version of real-time PCR, known as the Ruggedized Advanced Pathogen Identification Device (RAPID) system, has been developed for the rapid detection of *Escherichia coli* O157:H7. The rapid *E. coli* O157:H7 kit leverages the advantages of fast cycle real-time PCR, enabling accelerated and sensitive pathogen identification (60). An alternative method to traditional real-time PCR involves the use of three TaqMan assay sets targeting the *stx1*, *stx2*, and *rfbE* genes. This multiplex assay allows for highly specific detection of enterohemorrhagic *E. coli* (EHEC), including strains that may lack one or more of these key virulence markers. Results from such assays have also revealed horizontal gene transfer of the *stx* gene between *E. coli* strains and even across genera, such as into *Citrobacter* and *Enterobacter* species, highlighting the genetic mobility of these virulence factors and the complexity of pathogen detection (61).

All of the double-stranded DNA in the sample is instantly bound by the cyanine dye SYBR Green. During PCR, the target DNA sequence is amplified by the action of DNA polymerase, resulting in the generation of multiple copies of the desired DNA fragment. To monitor this amplification, SYBR Green dye is commonly used; it binds specifically to double-stranded DNA (62). As the PCR cycles progress and more double-

stranded DNA is produced, the fluorescence intensity of SYBR Green increases proportionally, allowing real-time quantification of the amplification process.

SYBR Green dye binds to all double-stranded DNA, hence the outcome is an increase in fluorescence intensity proportionate to the amount of PCR product produced. To identify *E. coli* strains, real-time PCR was employed in conjunction with the dye SYBR Green. The outcome demonstrated that the discriminating power between the strains was enhanced by the presence of SYBR Green (63). To identify In the case of *E. coli* O157:H7, a restriction site-specific PCR (RSS-PCR) method was employed. This technique involves amplifying DNA fragments using primers designed to target specific recognition sequences of restriction enzymes. The PCR amplification is tailored to produce fragments that are specific to the *E. coli* O157:H7 strain, facilitating its identification by the characteristic pattern of DNA fragments generated after restriction enzyme digestion.

Endonucleases are not used in this procedure. It produces amplicons that, when resolved on an agarose gel, produce "fingerprint" patterns. STEC was identified in both O157 and non-O157 serotypes of *E. coli* in cattle feces using multiplex PCR with SYBR Green (64).

Multiplex PCR detects *Salmonella* strains and *E. coli* O157:H7 simultaneously and semi-automatically using two sets of primers and two fluorogenic probes. In order to get a robust and consistent fluorescence signal from probes tagged with two reporter dyes, this PCR technique was optimised. This aided in the quick and precise identification of meat and excrement (56, 65). Fluorescence was combined with multiplex PCR and real-time PCR to facilitate the early detection of the *stx1, hly, and eae* genes. When the experiment was conducted under isothermal conditions, this resulted in a billion-fold amplification (66). LSplex, or large-scale multiplex, employs 800 distinct primer pairs. Both Gram-positive and Gram-negative pathogens can be effectively amplified by it. Compared to those that employed 2–5 µg of DNA, it produced greater signals using only 10 ng of DNA. The ability to lower the detection limit of LSplex PCR to pico (10−12g) or femtogrammes (10−15g) is one area that could be improved. When detecting any clinical, dietary, or environmental samples, this will be highly beneficial (67).

Hybridisation based on fluorescent amplification the most crucial factor in pathogen detection is fluorescence intensity, which PCR produces good findings for. *E. Coli* O157:H7 was found to have a fluorescence signal of 6.40, whereas other similar pathogens had a signal of 2.50. It's also reasonably priced. Another method that creates DNA from RNA using the reverse transcriptase enzyme is called reverse transcription PCR (RT-PCR), which is followed by the standard PCR procedure. This method is employed to identify the dengue virus (68). SYBR Green dye in RT-PCR has also been used to detect *Salmonella sp*. in samples of sausage and pork chops (69). When mRNA is discovered, real-time RT-PCR has demonstrated significant promise in identifying live infections like *S. enterica*. The expression of the *Salmonella*-specific sigDE operon, which codes for invasion proteins, was examined in one study, and it was discovered that the sigDE might be a practical and effective indicator for the bacteria (68).

IV. Discussion

Foodborne diseases continue to pose a major global public health threat, especially in developing regions where food handling and sanitation practices may be suboptimal. The increasing frequency and severity of outbreaks caused by pathogens like *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus* underscore the urgent need for robust detection systems. This review highlights the crucial role of early and accurate pathogen detection in preventing large-scale foodborne illnesses and protecting vulnerable populations such as infants, the elderly, and immune-compromised individuals.

Traditional microbiological methods like culture-based techniques, while still foundational, are time-consuming and less suited for rapid decision-making in public health crises. In contrast, immunological and molecular methods—especially ELISA and PCR-based techniques—offer greater sensitivity, specificity, and speed. ELISA-based assays, particularly sandwich ELISA, have demonstrated high accuracy in detecting specific foodborne pathogens and their toxins. Similarly, PCR and its variations (qPCR, multiplex PCR,

LAMP, and RT-PCR) have transformed the field of pathogen detection, enabling simultaneous, rapid, and high-throughput identification of multiple bacterial and viral pathogens with excellent precision.

Despite their advantages, each method has limitations. Immunoassays are susceptible to cross-reactivity, while PCR methods can yield false positives due to non-specific amplification and cannot distinguish between live and dead cells. Moreover, many of these methods require sophisticated equipment and trained personnel, which may limit their applicability in low-resource settings. Innovations such as biosensor integration, miniaturized lab-on-chip systems, and isothermal amplification technologies like LAMP are bridging these gaps by offering portability, affordability, and ease of use without compromising reliability.

The global prevalence of foodborne pathogens in a diverse range of food products—from dairy and meat to water and produce—emphasizes the need for standardized, universally accessible, and rapid detection methods. Emerging technologies are increasingly being tailored to meet these challenges, with a focus on field-deployable, cost-effective, and real-time detection systems.

V. Conclusion

Effective detection of foodborne pathogens is critical to safeguarding public health, ensuring food safety, and preventing outbreaks of disease. While traditional culture-based methods provide the basis for pathogen identification, rapid techniques such as immunological assays (e.g., ELISA) and molecular tools (e.g., PCR, qPCR, LAMP) have significantly improved the detection landscape by enabling quicker and more accurate results.

The continuing evolution of detection technologies—including multiplexing, real-time analysis, and point-of-care diagnostics—offers promising avenues for improving food safety across the globe. However, challenges such as cost, accessibility, and the risk of false results necessitate further refinement. A multipronged approach that integrates conventional, immunological, and molecular methods may provide the most comprehensive solution for timely detection, outbreak control, and risk assessment in the food industry and public health sectors. Continued research and investment in novel detection systems will be pivotal in achieving global food safety and reducing the burden of foodborne diseases.

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