Review of Contemporary Philosophy
ISSN: 1841-5261, e-ISSN: 2471-089X
Vol 22 (1), 2023
pp. 4241 - 4253

Innovative Advances in Polymerase Chain Reaction (Pcr) Techniques for The Enhanced Detection and Identification of Foodborne Pathogens: A Comprehensive Review

[1]-Fayz Eid Albuthely,[2]-Abdulrahman Mulla Alsaid ,[3]-Bakr Mohammed Hamoud Aljohani ,[4]-Raid Ghazi Alsulaimani ,[5]-Khalid Marzooq Aljohani ,[6]-Naif Snead Almrwani,[7]-Ali Eid Alhebaishi,[8]-Moayed Anwar Shahata,[9]-Hamed Abdullah Khalf Alharbi,[10]-Ghazi Ateyatullah Alrefai ,[11]-Naji Omear Mansour Aljohani

[1] Ksa, Ministry Of Health, Yanbu General Hospital
[2] Ksa, Ministry Of Health, Yanbu General Hospital
[3] Ksa, Ministry Of Health, Yanbu General Hospital
[4] Ksa, Ministry Of Health, Yanbu General Hospital
[5] Ksa, Ministry Of Health, Yanbu General Hospital
[6] Ksa, Ministry Of Health, Yanbu General Hospital
[7] Ksa, Ministry Of Health, Yanbu General Hospital
[8] Ksa, Ministry Of Health, Yanbu General Hospital
[9] Ksa, Ministry Of Health, Yanbu General Hospital
[10] Ksa, Ministry Of Health, Yanbu General Hospital
[11] Ksa, Ministry Of Health, Yanbu General Hospital

Abstract

Background: Microbial pathogens pose significant threats to food safety and public health, with foodborne illnesses affecting millions worldwide. Traditional detection methods often lack the sensitivity and speed required for effective monitoring. Recent advances in Polymerase Chain Reaction (PCR)-based techniques have revolutionized pathogen detection, offering rapid and specific identification of foodborne microorganisms.

Methods: This review synthesizes recent developments in PCR methodologies, including multiplex PCR, Real-Time PCR, Reverse Transcriptase PCR (RT-PCR), and Next Generation Sequencing (NGS). A comprehensive evaluation of these techniques was conducted, focusing on their application in identifying bacterial, fungal, and viral pathogens across various food matrices. The review also discusses the challenges associated with traditional culture-based methods and highlights the benefits of modern molecular techniques.

Results: The findings indicate that PCR-based methods significantly enhance the detection and quantification of foodborne pathogens compared to conventional techniques. Real-Time PCR demonstrated high sensitivity and specificity for pathogens such as *Salmonella*, *Listeria*, and *E. coli*, while NGS provided insights into the microbial composition of food samples, enabling the identification of complex pathogen communities.

Conclusion: The integration of advanced PCR techniques into food safety protocols is essential for mitigating the risks associated with foodborne pathogens. These methodologies not only improve detection rates but also facilitate timely public health responses to outbreaks. Future research should focus on optimizing these techniques for routine use in food safety laboratories and exploring novel approaches for pathogen control.

Keywords: Food safety, Pathogen detection, PCR techniques, Molecular biology, Foodborne illnesses.

Received: 10 october 2023 **Revised:** 24 November 2023 **Accepted:** 08 December 2023

1. Introduction

Microorganisms have a crucial role in food production, safety, and deterioration. Microbial interactions with food may render it unsuitable for human ingestion [1,2]. Some foodborne microbes not only lead to food spoiling but are also pathogenic, posing dangers to food safety and contributing to foodborne diseases among food handlers, customers, and society at large [3,4]. Foodborne illnesses and diseases pose significant hazards to human health and life, with more than 200 recognized foodborne infections. Prevalent foodborne pathogens include germs like *Campylobacter* species, *Bacillus cereus*, and *Clostridium botulinum*. *Clostridium perfringens*, some serogroups of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Shigella* species, and *Vibrio* species. and so forth [5-7]. As a result, detection techniques were established for these pathogens to guarantee adherence to established legal and regulatory food safety standards based on either the presence or absence of pathogens and their numbers. Foodborne fungus species, including *Penicillium*, *Claviceps*, *Aspergillus*, and *Fusarium*, pose a significant public health risk due to their potential to create mycotoxins [6]. Principal foodborne pathogenic viruses include Hepatitis A and E viruses, Norovirus, Rotavirus, Sapovirus, Enterovirus, Adenovirus, Astrovirus, and Achivirus [7,8].

Recently, substantial progress has been made in food preservation techniques aimed at prolonging shelf-life and eradicating spoilage and harmful microorganisms. Commonly employed methods encompass low-temperature storage (chilling and freezing), the application of chemical preservatives (e.g., essential oils and bacteriocins), and vacuum or modified atmosphere packaging [9-11]. Although certain methods eradicate food pathogens, research indicates that exposure to environmental or preservation-related stress may allow some pathogens to survive as metabolically injured cells with compromised metabolic functions. In such instances, these pathogens may either recover from the damage or remain irreversibly impaired [12-14]. They may also exist as dormant cells, such as viable but non-culturable cells (VNBC) or persister cells [15]. VNBC cells are non-culturable cells that exhibit low, detectable metabolic activity using established growth medium, while persister cells are surviving cells that are tolerant, but not resistant, to antibiotics, characterized by low metabolic activity and undetectable viability in experiments. Nonetheless, persister cells may become culturable when subjected to a particular stimulation [15].

The frequent incidence of foodborne infections indicates that, despite considerable progress in food production and safety, foodborne microorganisms remain substantial public health threats [15]. As a result, many technologies, both culture-dependent and culture-independent, have been developed to identify foodborne pathogens in food and safeguard public health. Numerous evaluations have been conducted on the detection of foodborne pathogens utilizing both culture-dependent and independent methodologies, including culture media, Polymerase Chain Reactions (PCR) such as multiplex PCR, Real-Time PCR, Reverse Transcriptase PCR (RT-PCR), DNA microarray, nucleic acid sequence-based amplification (NASBA), Next Generation Sequencing (NGS), as well as immunological and nanotechnology-based techniques [15-17].

Nonetheless, the majority of these evaluations mostly focus on bacteria, with just a cursory reference to other foodborne pathogenic groupings. Bacteria are the most prevalent foodborne pathogens of public health importance. Nonetheless, other pathogenic entities, such fungi and viruses, are significant since they are recognized for causing outbreaks of foodborne disorders. This review addresses bacteria, fungi, and viruses, emphasizing specific molecular techniques such as PCR (RT PCR, Multiplex PCR, End-point PCR, etc.) and NGS to enhance our understanding of detection and identification methods for various foodborne pathogens. The most current statistics on foodborne illness occurrences are provided, together with instances of recent outbreaks, to illustrate that foodborne pathogens remain a substantial threat to public health.

2. Incidences of Foodborne Illnesses

I. Bacteria

Bacterial foodborne diseases are a global phenomenon. In the USA, foodborne infections are predicted to result in between 128,000 and 325,000 hospitalizations, 3,000 to 5,000 deaths, and at least 76 million illnesses annually, incurring economic costs of up to $83 billion per year [4,18,19]. In the European Union in 2020, there were 20,017 human cases and 3,086 foodborne outbreaks of foodborne infections, with *Salmonella* (in eggs and egg products) identified as the predominant bacterial causal agent for these outbreaks. In addition to *Yersinia, Salmonella* and *Campylobacter*, Shiga toxin-producing *Escherichia coli* (STEC), and *Listeria monocytogenes* were the other predominant causal agents of foodborne infections in the EU in 2020. L. Illnesses associated with monocytogenes were among the leading causes of human mortality in the EU during this period [20-23]. Globally, it is estimated that around 3 million episodes of diarrhea attributable to foodborne microbial infections occur each year. The Australian Department of Health estimates that 4.1 million instances of foodborne infections occur in Australia. A review of foodborne microorganisms and their related diseases has been compiled by the authors [5].

II. Fungi

Unlike bacteria and viruses, outbreaks of infections linked to fungal foodborne pathogens are few, since only around 300 of the estimated 1.5 million fungal species are recognized as harmful to humans. Nonetheless, there are significant instances of sickness outbreaks, usually attributed to fungal secondary metabolites like toxins, sometimes affecting only susceptible groups, such as those with compromised immune, those who have had transplants, or those receiving immunosuppressive therapies. Nonetheless, seldom, some immunocompetent individuals may also fall victim to foodborne fungal infections.

In 2013, an epidemic of gastroenteritis (characterized by vomiting, nausea, and diarrhea) affected approximately 200 individuals in the USA who purchased yoghurts infected with *Mucor circinelloides*. Seven hospital patients in Hong Kong had an epidemic of food poisoning caused by Rhizopus microsporus from contaminated pre-packaged ready-to-eat meals or cornstarch used in the production of allopurinol tablets [24]. A literature study [25] indicates that some filamentous fungi, including *Aspergillus*, *Mucor*, and *Fusarium*, may induce localized infections in the sinuses, lungs, and other regions of immunocompetent individuals when ingested via contaminated food or breathed. Invasive pulmonary illness induced by some fungal infections often manifests in immunocompromised individuals, with inhalation being the predominant route of infection. Nevertheless, gastrointestinal pathways may also be significant, therefore necessitating that these individuals refrain from consuming foods potentially infected with fungus [25]. A comprehensive review of foodborne fungal agents was compiled by the authors [26].

III. Pathogenic agents

Viral food poisoning outbreaks are recorded less often than bacterial outbreaks. Noroviruses and Hepatitis A viruses are among the most prevalent foodborne viral infections responsible for human diseases. Noroviruses are associated with about 21 million episodes of acute gastroenteritis each year in the United States. In 2020, Norovirus in crustaceans, shellfish, molluscs, and their derivatives was a significant contributor to recorded foodborne outbreaks in the EU. Enteric viruses are believed to constitute around 13% and 45% of foodborne disease outbreaks in the EU and the US, respectively, with occasional public announcements of these outbreaks. A compilation of viral foodborne diseases has been documented by [8].

A recent analysis of documented virus-associated foodborne illness outbreaks has revealed that the gastroenteritis outbreak during the 2018 Winter Olympics in South Korea (194 cases), the frozen raspberry-related outbreak in Canada in 2017 (700 cases), and the outbreak aboard a Royal Caribbean cruise line in 2017 were all associated with Noroviruses [27]. Literature evaluations indicate that in 2014, viral agents constituted 20% of all documented outbreaks in the EU [20]. Notable instances are the 162 Hepatitis A virus infections in the United States in 2011 (due to the ingestion of tainted pomegranate seeds) and over 1100 cases in China in 2012 (Norovirus linked to frozen strawberries). In 2020, diseases caused by the West Nile virus were among the predominant foodborne pathogens leading to the largest number of human deaths in the EU [20].

3. Techniques for Identifying Foodborne Pathogens

The identification of pathogens in food matrices with high repeatability and sensitivity is crucial for ensuring food safety. Nonetheless, it remains a formidable problem owing to variables such as interference from other and non-target microbiota, limited populations of target groups, and difficulties in microbial extraction from food matrices. Various techniques are used for the detection and identification of foodborne pathogens. These include culture-based techniques, immunological tests, and nucleic acid-based procedures, including Polymerase Chain Reactions (PCR) and Next Generation Sequencing (NGS). This study offers a concise elucidation of the concepts behind each category of method (excluding biosensors), when applicable, followed by an examination of the application of many commonly used techniques for pathogen detection in contemporary literature. This study examines the use of culture-based techniques, immunological tests, PCR, and Next Generation Sequencing methodologies [28].

The efficacy of any of these methods relies on the implementation of suitable aseptic sampling and sample storage techniques. The sampling strategy relies on the kind of food being analyzed, the specific microbial groups targeted, and the tools used for microbial detection. Sample collection and analysis for methods involving the isolation and use of microbial cultures must adhere to standard standards established by authoritative organizations like as the FDA, FSIS/USDA, ISO, and AOAC [29]. Standardized procedures are available for sample collection, analysis, and data interpretation, contingent upon the specifications provided by the kit, reagent, and equipment manufacturers.

I. Culture-based Methods

Culture-based techniques continue to serve as the standard for identifying foodborne pathogens, despite the availability of more contemporary approaches [30-33]. These approaches rely on the capacity of bacteria and fungi to proliferate on the culture medium, resulting in visible colonies that may then undergo further tests as necessary. They continue to be the preferred option and may be mandated by legislation for the identification of microorganisms in food testing facilities [15]. They may be used to provide culture-dependent qualitative and quantitative data on the presence of foodborne pathogens. Culture-based techniques are more effective when the growth needs of target microorganisms are understood, using culture medium to enrich, selectively isolate, or differentiate between target microorganisms and other groups [6]. Furthermore, cultures may undergo assessments including colony morphology, Gram staining, biochemical characterization, MALDI TOF MS, and PCR sequencing for identification purposes [6].

Notwithstanding its user-friendliness, affordability, and capacity to isolate microorganisms for further applications, culture-based approaches possess considerable limitations. These approaches exhibit limited sensitivity as not all microorganisms are culturable, and some culturable bacteria, such as *S. typhi* and *E. coli*, may persist in a viable but non-culturable (VBNC) state due to stress and other conditions [15,34]. This may result in the underestimation or failure to identify foodborne microorganisms, hence posing a possible danger to food safety [35]. The cultivation of microorganisms is a protracted and arduous procedure that requires many phases and may need supplementary techniques (e.g., biochemical, serological, nucleic acid-based procedures for definitive identification), potentially taking up to a week for bacteria or longer for fungus. They may also be inadequate for the rapid identification of microorganisms for immediate or real-time detection of foodborne pathogens [36,37].

II. Immunological Assessments

These tests include serotyping, immunofluorescence, lateral flow devices (LFD), and enzyme-linked immunosorbent assay (ELISA), with ELISA being a very precise immunological technique for the detection of foodborne pathogens and their toxins. These methodologies operate on the notion of the affinity between microbial antigens and antibodies, which may be leveraged for the fast and precise identification of foodborne pathogens [37]. The primary benefits of these tests are their simplicity, rapidity compared to culture-based approaches, ability to identify poisons, and high specificity. The contamination of the reaction matrix may result in the production of false positives [38]. ELISA and lateral flow devices (LFD) are among the most prevalent immunoassays for the detection of foodborne microorganisms and their toxins, with their principles and mechanisms of action thoroughly reviewed [16,38,39]. ELISA may occasionally be employed alongside other techniques, such as PCR, to enhance specificity and efficiency. A PCR-ELISA method has resulted in a 100-fold enhancement in the identification of *Fusarium verticillioides* in infected maize samples relative to traditional PCR detection [40].

III. Nucleic Acid-Based Techniques (Polymerase Chain Reaction (PCR) and Its Variants (e.g., RT-PCR))

PCR and its derivatives are nucleic acid-based techniques used to identify particular DNA or RNA sequences of harmful bacteria. Targeted primers are engineered for specific diseases, enabling their exponential amplification in food samples. PCR-based methods are more rapid, sensitive, repeatable, and adaptable than most culture-based and immunoassay techniques, establishing them as the preferred alternative for detecting foodborne pathogens. They are used on nucleic acids derived from food samples or microbiological isolates obtained from food samples [16,38,41,42].

This nucleic acid-based technique has several versions (Figure 1). This encompasses traditional PCR techniques (nested, touchdown, hot-start PCR, etc.), which are often used for the detection of foodborne pathogens using primers that target the DNA of these pathogens. Nested PCR is an adaptation of conventional PCR that employs two distinct primer sets throughout two separate PCR reaction cycles. The second primer set amplifies a secondary target using the amplicon produced by the first primer set [41]. Nested PCR is specifically engineered to detect minimal concentrations of target pathogens [43]. Touchdown PCR inhibits the amplification of non-specific sequences. This is accomplished by initiating the early PCR steps at elevated annealing temperatures, followed by gradual reductions in succeeding cycles, so enabling the primer to bind to the target sequence at the maximum temperature that inhibits the amplification of non-specific sequences. Multiplex PCR is a version that enables the simultaneous detection of numerous diseases by using various primers within a single reaction [41].

Reverse Transcriptase PCR (RT-PCR) facilitates the identification of RNA (metabolically active microorganisms) by synthesizing complementary DNA (cDNA) from the RNA transcript, followed by the amplification of the cDNA. Real-time or quantitative PCR (qPCR) quantifies pathogen load on food products by fluorescent dyes or probes, utilizing a specifically constructed thermal cycler. It contrasts with traditional PCR by monitoring the amplification of target DNA in real-time rather than at the endpoint, as is the case with conventional PCR. RT-PCR and qPCR may be integrated (RT-qPCR) for both qualitative and quantitative applications. RNA undergoes reverse transcription to become cDNA, which is then analyzed using Real-Time PCR for quantitative RNA detection [41]. Alternative PCR methodologies, such as droplet digital PCR (ddPCR), provide quantification without necessitating a standard curve [44,45]. In DDPCR, target samples are divided into thousands of droplets, each consisting of one-nanolitre reverse micelles (water in oil), which undergo a fluorescence probe-based PCR experiment. Loop-mediated isothermal amplification (LAMP) is a DNA amplification technique that produces around one billion copies of DNA, surpassing the one million copies generated by conventional PCR, all within one hour [46]. The process employs a maximum of three primer pairs to target up to eight particular loci on DNA strands during amplification processes performed at consistent temperatures [46].

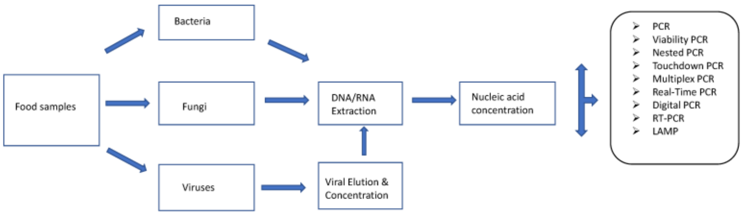

Figure 1. Methods using nucleic acids for the detection and identification of foodborne diseases [47].

Identification of Foodborne Bacteria Utilizing PCR-Based Techniques

Conventional PCR and its derivatives, which are DNA-based, use primers (specific or degenerate) to identify a target or group of bacteria in food products. Nonetheless, these methodologies (endpoint PCR, nested PCR, multiplex PCR, etc.) are incapable of determining the viability of the target organism(s). These methodologies have been effectively used to identify significant bacterial pathogens, including *Salmonella* spp., *Campylobacter* sp., and *Escherichia coli* O157:H7, *Salmonella*. *Staphylococcus aureus*, *B. cereus*, *L. monocytogenes* and *V. parahaemolyticus* are present in ready-to-eat Korean meals, Minas Frescal cheese, milk, beef, contaminated chicken eggs, pork, salad, and seafood [48-53].

Real-Time or quantitative PCR is more prevalent than traditional PCR or multiplex PCR for detecting and quantifying the prevalence of significant foodborne bacterial pathogens in extracts from food samples or purified isolates. *Salmonella*, *Escherichia coli*, *Campylobacter*, and *Listeria monocytogenes* have been identified and quantified in cheese, poultry, beef burgers, turkey, pig, eggs, and fish via Real-Time PCR [34,54-56]. Employing the same technique, *Clostridium perfringens*, *Escherichia coli*, and *Staphylococcus aureus* were identified and measured in fresh and ready-to-eat vegetables [57]. Pathogens including *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae* have been identified in seafood, shrimp, and mussels by Real-Time PCR [58,59].

Conversely, Reverse Transcriptase PCR (RT-PCR) technologies are less prevalent for the detection of foodborne bacterial infections compared to Real-Time or quantitative PCR. This is because, while mRNA serves as a superior signal of bacterial vitality, it is swiftly destroyed inside the food matrix, leading to false negative findings [60-62]. The technique is labor-intensive, and these reasons explain why this methodology is rarely used for identifying foodborne germs compared to other approaches. Nonetheless, this method has been used to identify bacterial pathogens in several food samples, including *S. Typhimurium* in intentionally contaminated jalapeño and serrano peppers and *S. enterica* in spiked egg broth and milk [63,64].

Viable PCR (vPCR) is a type of PCR that employs intercalating dyes, such as ethidium monoazide (EMA) and propidium monoazide (PMA), for sample pre-treatment, followed by PCR amplification to identify viable foodborne pathogens [65]. vPCR may be more effectively used for pathogen identification compared to RT PCR. These intercalating dyes may infiltrate the membranes of compromised or deceased bacterial cells, permanently attaching to their DNA molecules and so obstructing amplification by PCR primers. Consequently, any amplicon detected in the following PCR will originate from viable bacterial cells [65]. It has been used to identify live *Salmonella* species. In spiked pig meat and ready-to-eat salad, as well as *Helicobacter pylori* in retail pork meat, this technique is effective in evaluating food safety [66-68]. The integration of vPCR with Real-Time PCR has facilitated the precise identification and quantification of live Campylobacter species. in frozen and refrigerated poultry products [69] and *Listeria* species. in spiked meat, salad, cheese, and milk. Alternative nucleic acid amplification techniques, including LAMP (Loop-Mediated Isothermal Amplification), have been used to identify *V. vulnificus* and *V. parahaemolyticus* in food products, particularly seafood [70].

Identification of Foodborne Fungi Utilizing PCR-Based Techniques

A literature study revealed a paucity of studies about the identification of pathogenic fungi on food products by PCR-based approaches, in contrast to bacteria. Numerous findings indicate the identification of fungal infections in clinical and environmental samples using nucleic acid and PCR-based techniques [71,72]. Various PCR or nucleic acid-based techniques, including endpoint PCR, real-time PCR, nested PCR, quantitative (RT)-PCR, loop-mediated isothermal amplification (LAMP), and multiplex PCR, have been used for the identification of pathogenic or mycotoxigenic fungus [73]. These approaches have been used directly on extracts from food samples, swabs of food surfaces, and isolates from food samples to identify and characterize foodborne fungal pathogens.

Multiplex PCR has been devised and effectively used to identify aflatoxigenic *Aspergillus* isolates from "meju," a traditional fermented soybean food starter from Korea [74]. In this case, specifically engineered primers were used to effectively differentiate between aflatoxin-producing and non-aflatoxin-producing fungi, as corroborated by TLC and HPLC analyses of filtrates from test cultures [75]. Multiplex PCR has been used to concurrently identify Aspergillus, Penicillium, and Fusarium in contaminated maize grain powder [76].

Patulin-producing *Penicillium expansum* has been effectively identified on intentionally infected apples using RT-PCR [77]. Loop-mediated isothermal amplification (LAMP) tests have been established for the rapid identification of ochratoxin-producing Penicillium nordicum in dry-cured pork products [78]. PCR, in conjunction with techniques like denaturing gradient gel electrophoresis (DGGE) and sequencing, is now capable of detecting ochratoxin-producing Aspergillus niger in wine [79]. DGGE is a technique used to segregate DNA fragments (PCR amplicons) according to their melting properties on polyacrylamide gels. This produces a gel-based fingerprint of the main microbial groups, and further techniques may be used to describe the identification of these groups if necessary [79].

Identification of Foodborne Viruses Utilizing PCR-Based Techniques

Molecular techniques include PCR, Multiplex PCR, Real-Time PCR (RT-PCR), digital RT-PCR, and Quantitative RT-PCR may be used to identify viral infections in food. RT-PCR is a widely used technique, perhaps due to the prevalence of RNA viruses and its efficacy in detecting foodborne viral infections. This is due to its capacity to quantify viral particles when used with quantitative real-time PCR, which is highly sensitive, specific, and capable of high throughput [27]. These may be directly used to food samples or combined with a cell culture test (isolation of pathogens by cell culture followed by detection using PCR) [57]. Viruses must be isolated and concentrated from food components prior to the use of PCR. The technique's shortcomings include potentially inefficient extraction techniques, leading to minimal recovery or complete loss of viral particles, and the amplification process may be hindered by sample inhibitors. RT PCR, or real-time quantitative PCR (RT qPCR), cannot differentiate between infected and non-infectious particles [59]. To address the limitations in detecting infectious particles, intercalating dyes like propidium monoazide (PMA) or ethidium monoazide (EMA) can be employed for sample pre-treatment prior to RT PCR or RT qPCR, thereby inhibiting amplification from non-infectious particles. This method has effectively differentiated infectious Hepatitis A viruses and Rotavirus from non-infectious particles in laboratory analyses [80].

Numerous instances exist of RT-PCR used for virus identification in food. The incidence of Hepatitis A and Norovirus was examined in harvested mussels in Italy. RT-PCR was used to unambiguously demonstrate the significant incidence of norovirus, highlighting the public health concern linked to mussel eating [81]. RT-PCR has also been used to identify zoonotic Hepatitis E viruses in contaminated pig liver sausages [126] and in uncooked and liver sausages available in retail outlets in Germany. This methodology was used in Brazil to evaluate the incidence of Adenovirus, Hepatitis E virus, and Rotavirus in beef, pig, and chicken meat cuts from a local market, revealing that Rotavirus was the predominant viral pathogen in the chicken samples [82].

RT-PCR has been used in conjunction with novel techniques such as microfluidics to identify viruses in various food products or to improve the viral detection efficacy of other molecular approaches. Unprocessed fruits, particularly soft berries, may contain viruses like Norovirus and Hepatitis A virus, which are responsible for gastroenteritis. Nonetheless, their concentrations and infectious dosages are often minimal in contaminated food samples. Consequently, microfluidics was effectively used to improve the detection of these two viruses in soft berries by digital RT-PCR, resulting in reduced PCR inhibition and increased viral detection efficiency [83]. Microfluidic digital PCR effectively detected Norovirus and Hepatitis A viruses in contaminated lettuce, demonstrating much greater viral recoveries compared to other molecular techniques as RT-PCR [84].

IV. Methods of Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) methodologies combined with bioinformatics are potent techniques that have significantly improved food safety. Next-generation sequencing (NGS) is mostly used for two purposes: firstly, to ascertain the whole genome sequence of an isolate (whole genome sequencing or WGS), and secondly, in metagenomics, to identify the sequences of various microbes present in a sample. In this application, specific microbial groupings (bacteria, fungi, or viruses) may be identified using 16S rRNA, ITS, or other biological markers [85]. Figure 2 illustrates the various processes of next-generation sequencing (NGS) used in food microbiology. Next Generation Sequencing approaches are well-reviewed about their concepts, kinds, benefits, and limitations [39,40].

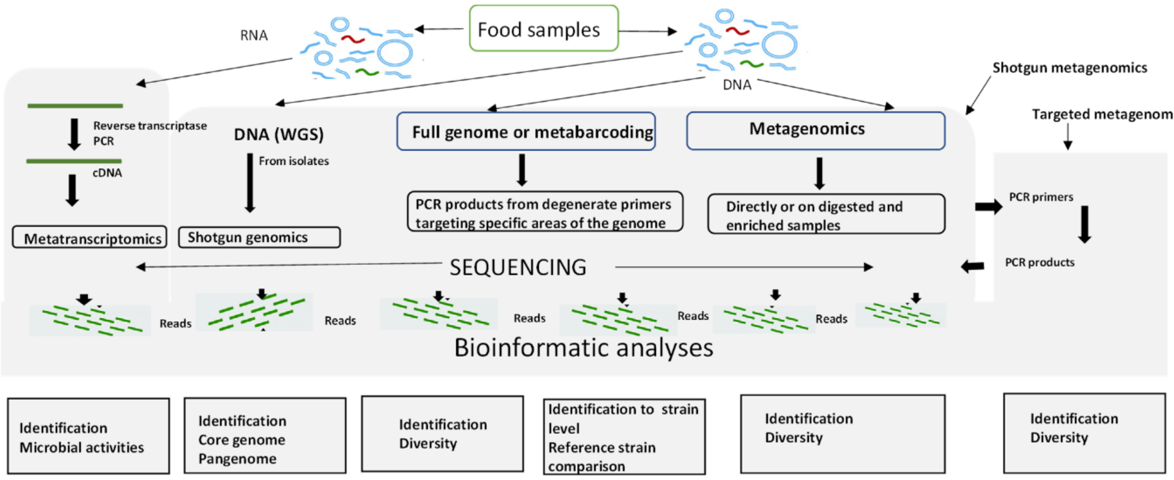

Figure 2. Next-generation sequencing methodology for the detection and identification of foodborne pathogens [32].

4. Conclusions and Prospective Directions

A review of four techniques was conducted: culture-based, immunoassay, nucleic-acid-based (PCR), and NGS-based procedures. Culture-based methods, when combined with techniques such as MALDI TOF MS and nucleic acid-based methods like PCR (Real-Time PCR for bacteria and RT-PCR for fungi), are effective for the rapid detection and identification of various foodborne pathogens, particularly those within bacterial and fungal categories. Nucleic acid-based techniques, including Real-Time PCR and vPCR in conjunction with sequencing technologies, are more prevalent than immunoassays and NGS-based methods for pathogen identification. NGS-based methodologies, including metagenomics, provide exceptional insights into the genotype, diversity, and activity of foodborne pathogens, facilitating pathogen monitoring, tracking, screening within the food chain, and identification. These methods would significantly aid in the early diagnosis and management of foodborne outbreaks, ultimately improving public safety and health. Current and future initiatives should concentrate on the use of current technologies for the control of foodborne pathogens by educating food surveillance and safety personnel in these methodologies. Furthermore, further study is necessary to create more effective strategies, such as using bacteriophages for the control and eradication of foodborne infections.

References

[1] Saucier, L. Microbial spoilage, quality and safety within the context of meat sustainability. Meat Sci. 2016, 120, 78–84.

[2] Iulietto, M.F.; Sechi, P.; Borgogni, E.; Cenci-Goga, B.T. Meat spoilage: A critical review of a neglected alteration due to ropy slime producing bacteria. Ital. J. Anim. Sci. 2015, 14, 4011.

[3] Quthama, A.-Z.; Diguţă, C.F.; Dopcea, G.; Matei, F. Conventional Versus Modern Techniques Used for the Detection of Pathogens in Food Matrices: A Review. Sci. Bull. Ser. F. Biotechnol. 2022, 26, 79–88.

[4] Mead, P.S.; Slutsker, L.; Dietz, V.; McCaig, L.F.; Bresee, J.S.; Shapiro, C.; Griffin, P.M.; Tauxe, R.V. Food-related illness and death in the United States. Emerg. Infect. Dis. 1999, 5, 607.

[5] Bintsis, T. Foodborne pathogens. AIMS Microbiol. 2017, 3, 529.

[6] Martinović, T.; Andjelković, U.; Gajdošik, M.Š.; Rešetar, D.; Josić, D. Foodborne pathogens and their toxins. J. Proteom. 2016, 147, 226–235.

[7] Lianou, A.; Panagou, E.Z.; Nychas, G.-J.E. Meat safety—I foodborne pathogens and other biological issues. In Lawrie's Meat Science; Elsevier: Cambridge, UK, 2023; pp. 549–590.

[8] Pexara, A.; Govaris, A. Foodborne viruses and innovative non-thermal food-processing technologies. Foods 2020, 9, 1520.

[9] Xiong, Y.; Chen, M.; Warner, R.D.; Fang, Z. Incorporating nisin and grape seed extract in chitosan-gelatine edible coating and its effect on cold storage of fresh pork. Food Control 2020, 110, 107018.

[10] Ribeiro-Santos, R.; Andrade, M.; de Melo, N.R.; Sanches-Silva, A. Use of essential oils in active food packaging: Recent advances and future trends. Trends Food Sci. Technol. 2017, 61, 132–140.

[11] Batiha, G.E.-S.; Hussein, D.E.; Algammal, A.M.; George, T.T.; Jeandet, P.; Al-Snafi, A.E.; Tiwari, A.; Pagnossa, J.P.; Lima, C.M.; Thorat, N.D. Application of natural antimicrobials in food preservation: Recent views. Food Control 2021, 126, 108066.

[12] Falleh, H.; Jemaa, M.B.; Saada, M.; Ksouri, R. Essential oils: A promising eco-friendly food preservative. Food Chem. 2020, 330, 127268.

[13] Wang, T.; Guo, H.; Zhang, H.; Ren, F.; Zhang, M.; Ge, S.; Luo, H.; Zhao, L. Dynamics of Bacterial Communities of Lamb Meat Packaged in Air and Vacuum Pouch during Chilled Storage. Food Sci. Anim. Resour. 2019, 39, 209.

[14] Kaur, M.; Shang, H.; Tamplin, M.; Ross, T.; Bowman, J.P. Culture-dependent and culture-independent assessment of spoilage community growth on VP lamb meat from packaging to past end of shelf-life. Food Microbiol. 2017, 68, 71–80.

[15] Foddai, A.C.; Grant, I.R. Methods for detection of viable foodborne pathogens: Current state-of-art and future prospects. Appl. Microbiol. Biotechnol. 2020, 104, 4281–4288.

[16] Law, J.W.-F.; Ab Mutalib, N.-S.; Chan, K.-G.; Lee, L.-H. Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. Front. Microbiol. 2015, 5, 770.

[17] Saravanan, A.; Kumar, P.S.; Hemavathy, R.; Jeevanantham, S.; Kamalesh, R.; Sneha, S.; Yaashikaa, P. Methods of detection of food-borne pathogens: A review. Environ. Chem. Lett. 2021, 19, 189–207.

[18] Nyachuba, D.G. Foodborne illness: Is it on the rise? Nutr. Rev. 2010, 68, 257–269.

[19] Ribot, E.M.; Hise, K.B. Future challenges for tracking foodborne diseases. EMBO Rep. 2016, 17, 1499–1505.

[20] Authority, E.F.S. The European Union One Health 2020 Zoonoses Report. EFSA J. 2021, 19, e06971.

[21] Kirk, M.; Glass, K.; Ford, L.; Brown, K.; Hall, G. Foodborne illness in Australia: Annual incidence circa 2010. Canberra Aust. Gov. Dep. Health 2014, 20, 1857–1864.

[22] Hawksworth, D.L. The magnitude of fungal diversity: The 1· 5 million species estimate revisited. Mycol. Res. 2001, 105, 1422–1432.

[23] Lee, S.C.; Billmyre, R.B.; Li, A.; Carson, S.; Sykes, S.M.; Huh, E.Y.; Mieczkowski, P.; Ko, D.C.; Cuomo, C.A.; Heitman, J. Analysis of a food-borne fungal pathogen outbreak: Virulence and genome of a Mucor circinelloides isolate from yogurt. MBio 2014, 5, e01390-14.

[24] Cheng, V.C.; Chan, J.F.; Ngan, A.H.; To, K.K.; Leung, S.; Tsoi, H.; Yam, W.; Tai, J.W.; Wong, S.S.; Tse, H. Outbreak of intestinal infection due to Rhizopus microsporus. J. Clin. Microbiol. 2009, 47, 2834–2843.

[25] Paterson, R.R.M.; Lima, N. Filamentous fungal human pathogens from food emphasising Aspergillus, Fusarium and Mucor. Microorganisms 2017, 5, 44.

[26] Benedict, K.; Chiller, T.M.; Mody, R.K. Invasive fungal infections acquired from contaminated food or nutritional supplements: A review of the literature. Foodborne Pathog. Dis. 2016, 13, 343–349.

[27] Miranda, R.C.; Schaffner, D.W. Virus risk in the food supply chain. Curr. Opin. Food Sci. 2019, 30, 43–48.

[28] Yeargin, T.; Gibson, K. Key characteristics of foods with an elevated risk for viral enteropathogen contamination. J. Appl. Microbiol. 2019, 126, 996–1010.

[29] Da Silva, N.; Taniwaki, M.H.; Junqueira, V.C.; Silveira, N.; Okazaki, M.M.; Gomes, R.A.R. Microbiological Examination Methods of Food and Water: A Laboratory Manual; CRC Press: Boca Raton, FL, USA, 2018.

[30] Patil-Joshi, A.; Rangaswamy, B.; Apte-Deshpande, A. based PCR method development, validation and application for microbial detection. J. Genet. Eng. Biotechnol. 2021, 19, 1–10.

[31] Park, D.G.; Ha, E.S.; Kang, B.; Choi, I.; Kwak, J.E.; Choi, J.; Park, J.; Lee, W.; Kim, S.H.; Kim, S.H.; et al. Development and evaluation of a next-generation Sequencing panel for the multiple detection and identification of pathogens in fermented foods. J. Microbiol. Biotechnol. 2023, 33, 83–95.

[32] Altayb, H.N.; Badri, R.M.; Chaieb, K.; Moglad, E. Detection and characterization of the most common foodborne pathogens by using multiplex PCR procedure. Saudi J. Biol. Sci. 2023, 1–14.

[33] Chen, K.; Ma, B.; Li, J.; Chen, E.; Xu, Y.; Yu, X.; Sun, C.; Zhang, M. A rapid and sensitive europium nanoparticle-based lateral flow immunoassay combined with recombinase polymerase amplification for simultaneous detection of three food-borne pathogens. Int. J. Environ. Res. Public Health 2021, 18, 4574.

[34] Gill, A. The importance of bacterial culture to food microbiology in the age of genomics. Front. Microbiol. 2017, 8, 777.

[35] Zhao, X.; Lin, C.-W.; Wang, J.; Oh, D.H. Advances in rapid detection methods for foodborne pathogens. J. Microbiol. Biotechnol. 2014, 24, 297–312.

[36] Tietjen, M.; Fung, D.Y. Salmonellae and food safety. Crit. Rev. Microbiol. 1995, 21, 53–83.

[37] Oluwaseun, A.C.; Phazang, P.; Sarin, N.B. Biosensors: A fast-growing technology for pathogen detection in agriculture and food sector. In Biosensing Technologies for the Detection of Pathogens—A Prospective Way for Rapid Analysis, 1st ed.; INTECH: Jakarta Selatan, Indonesia, 2018; pp. 37–52.

[38] Priyanka, B.; Patil, R.K.; Dwarakanath, S. A review on detection methods used for foodborne pathogens. Indian J. Med. Res. 2016, 144, 327.

[39] Agriopoulou, S.; Stamatelopoulou, E.; Varzakas, T. Advances in analysis and detection of major mycotoxins in foods. Foods 2020, 9, 518.

[40] Omori, A.M.; Ono, E.Y.S.; Bordini, J.G.; Hirozawa, M.T.; Fungaro, M.H.P.; Ono, M.A. Detection of Fusarium verticillioides by PCR-ELISA based on FUM21 gene. Food Microbiol. 2018, 73, 160–167.

[41] Singh, J.; Birbian, N.; Sinha, S.; Goswami, A. A critical review on PCR, its types and applications. Int. J. Adv. Res. Biol. Sci. 2014, 1, 65–80.

[42] Muhamad Rizal, N.S.; Neoh, H.-m.; Ramli, R.; A/LK Periyasamy, P.R.; Hanafiah, A.; Abdul Samat, M.N.; Tan, T.L.; Wong, K.K.; Nathan, S.; Chieng, S. Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: Perspectives from a middle-income country. Diagnostics 2020, 10, 816.

[43] Mancini, V.; Murolo, S.; Romanazzi, G. Diagnostic methods for detecting fungal pathogens on vegetable seeds. Plant Pathol. 2016, 65, 691–703.

[44] Olmedillas-López, S.; Olivera-Salazar, R.; García-Arranz, M.; García-Olmo, D. Current and emerging applications of droplet digital PCR in oncology: An updated review. Mol. Diagn. Ther. 2021, 21, 493–510.

[45] Yang, R.; Paparini, A.; Monis, P.; Ryan, U. Comparison of next-generation droplet digital PCR (ddPCR) with quantitative PCR (qPCR) for enumeration of Cryptosporidium oocysts in faecal samples. Int. J. Parasitol. 2014, 44, 1105–1113.

[46] Soroka, M.; Wasowicz, B.; Rymaszewska, A. Loop-Mediated Isothermal Amplification (LAMP): The Better Sibling of PCR? Cells 2021, 10, 1931.

[47] Bosch, A.; Pintó, R.M.; Guix, S. Foodborne viruses. Curr. Opin. Food Sci. 2016, 8, 110–119.

[48] Lee, N.; Kwon, K.Y.; Oh, S.K.; Chang, H.-J.; Chun, H.S.; Choi, S.-W. A multiplex PCR assay for simultaneous detection of Escherichia coli O157: H7, Bacillus cereus, Vibrio parahaemolyticus, Salmonella spp., Listeria monocytogenes, and Staphylococcus aureus in Korean ready-to-eat food. Foodborne Pathog. Dis. 2014, 11, 574–580.

[49] Carvalho, R. Escherichia coli O157: H7 identification in Minas Frescalcheesecommercialized in Goiânia. GO. Braz. 2014, 45, 7–10. [Google Scholar]

[50] Moezi, P.; Kargar, M.; Doosti, A.; Khoshneviszadeh, M. Multiplex touchdown PCR assay to enhance specificity and sensitivity for concurrent detection of four foodborne pathogens in raw milk. J. Appl. Microbiol. 2019, 127, 262–273.

[51] Samad, A.; Abbas, F.; Ahmad, Z.; Tanveer, Z.; Ahmad, I.; Patching, S.G.; Nawaz, N.; Asmat, M.T.; Raziq, A.; Naeem, M. Multiplex polymerase chain reaction detection of Shiga toxin genes and antibiotic sensitivity of Escherichia coli O157: H7 isolated from beef meat in Quetta, Pakistan. J. Food Saf. 2018, 38, e12540.

[52] Vinayaka, A.C.; Ngo, T.A.; Kant, K.; Engelsmann, P.; Dave, V.P.; Shahbazi, M.-A.; Wolff, A.; Bang, D.D. Rapid detection of Salmonella enterica in food samples by a novel approach with combination of sample concentration and direct PCR. Biosens. Bioelectron. 2019, 129, 224–230.

[53] Pires, N.M.; Dong, T.; Yang, Z.; da Silva, L.F. Recent methods and biosensors for foodborne pathogen detection in fish: Progress and future prospects to sustainable aquaculture systems. Crit. Rev. Food Sci. Nutr. 2021, 61, 1852–1876.

[54] Bai, X.; Wang, Z.; Li, W.; Xiao, F.; Huang, J.; Xu, Q.; Xu, H. Rapid and accurate detection for Listeria monocytogenes in milk using ampicillin-mediated magnetic separation coupled with quantitative real-time PCR. Microchem. J. 2022, 183, 108063.

[55] Chin, N.A.; Salihah, N.T.; Shivanand, P.; Ahmed, M.U. Recent trends and developments of PCR-based methods for the detection of food-borne Salmonella bacteria and Norovirus. J. Food Sci. Technol. 2021, 59, 4570–4582.

[56] Heo, E.J.; Kim, H.-Y.; Suh, S.H.; MOON, J.S. Comparison of DNA Extraction Methods for the Quantification of Listeria monocytogenes in Dairy Products by Real-Time Quantitative PCR. J. Food Prot. 2022, 85, 1531–1537.

[57] Azimirad, M.; Nadalian, B.; Alavifard, H.; Panirani, S.N.; Bonab, S.M.V.; Azimirad, F.; Gholami, F.; Jabbari, P.; Yadegar, A.; Busani, L. Microbiological survey and occurrence of bacterial foodborne pathogens in raw and ready-to-eat green leafy vegetables marketed in Tehran, Iran. Int. J. Hyg. Environ. Health 2021, 237, 113824.

[58] Cao, X.; Zhao, L.; Zhang, J.; Chen, X.; Shi, L.; Fang, X.; Xie, H.; Chang, Y.; Wang, L. Detection of viable but nonculturable Vibrio parahaemolyticus in shrimp samples using improved real-time PCR and real-time LAMP methods. Food Control 2019, 103, 145–152.

[59] Bonnin-Jusserand, M.; Copin, S.; Le Bris, C.; Brauge, T.; Gay, M.; Brisabois, A.; Grard, T.; Midelet-Bourdin, G. Vibrio species involved in seafood-borne outbreaks (Vibrio cholerae, V. parahaemolyticus and V. vulnificus): Review of microbiological versus recent molecular detection methods in seafood products. Crit. Rev. Food Sci. Nutr. 2019, 59, 597–610.

[60] Yang, X.; Zhang, X.; Wang, Y.; Shen, H.; Jiang, G.; Dong, J.; Zhao, P.; Gao, S. A real-time recombinase polymerase amplification method for rapid detection of Vibrio vulnificus in seafood. Front. Microbiol. 2020, 11, 586981.

[61] Xiao, L.; Zhang, L.; Wang, H.H. Critical issues in detecting viable Listeria monocytogenes cells by real-time reverse transcriptase PCR. J. Food Prot. 2012, 75, 512–517.

[62] Miller, N.D.; Draughon, F.A.; D'Souza, D.H. Real-time reverse-transcriptase–polymerase chain reaction for Salmonella enterica detection from jalapeno and serrano peppers. Foodborne Pathog. Dis. 2010, 7, 367–373.

[63] Zhou, M.; Yang, J.; Zhou, X.; Liu, B.; Liu, D.; Yuan, C.; He, Y.; Pan, L.; Shi, X. Development of a sigDE-based real-time reverse-transcriptase PCR for the detection of viable Salmonella enterica. Foodborne Pathog. Dis. 2014, 11, 537–544.

[64] Chen, M.; Lan, X.; Zhu, L.; Ru, P.; Xu, W.; Liu, H. PCR Mediated Nucleic Acid Molecular Recognition Technology for Detection of Viable and Dead Foodborne Pathogens. Foods 2022, 11, 2675.

[65] Dinh Thanh, M.; Agustí, G.; Mader, A.; Appel, B.; Codony, F. Improved sample treatment protocol for accurate detection of live Salmonella spp. in food samples by viability PCR. PLoS ONE 2017, 12, e0189302.

[66] De Cooman, L.; Flahou, B.; Houf, K.; Smet, A.; Ducatelle, R.; Pasmans, F.; Haesebrouck, F. Survival of Helicobacter suis bacteria in retail pig meat. Int. J. Food Microbiol. 2013, 166, 164–167.

[67] Castro, A.G.; Dorneles, E.M.; Santos, E.L.; Alves, T.M.; Silva, G.R.; Figueiredo, T.C.; Assis, D.C.; Lage, A.P.; Cançado, S.V. Viability of Campylobacter spp. in frozen and chilled broiler carcasses according to real-time PCR with propidium monoazide pretreatment. Poult. Sci. 2018, 97, 1706–1711.

[68] Agustí, G.; Fittipaldi, M.; Codony, F. Optimization of a viability PCR method for the detection of Listeria monocytogenes in food samples. Curr. Microbiol. 2018, 75, 779–785.

[69] Sexton, D.J.; Kordalewska, M.; Bentz, M.L.; Welsh, R.M.; Perlin, D.S.; Litvintseva, A.P. Direct detection of emergent fungal pathogen Candida auris in clinical skin swabs by SYBR green-based quantitative PCR assay. J. Clin. Microbiol. 2018, 56, e01337-18.

[70] Wagner, K.; Springer, B.; Pires, V.; Keller, P.M. Molecular detection of fungal pathogens in clinical specimens by 18S rDNA high-throughput screening in comparison to ITS PCR and culture. Sci. Rep. 2018, 8, 6964.

[71] Luchi, N.; Ioos, R.; Santini, A. Fast and reliable molecular methods to detect fungal pathogens in woody plants. Appl. Microbiol. Biotechnol. 2020, 104, 2453–2468.

[72] Vergidis, P.; Moore, C.B.; Novak-Frazer, L.; Rautemaa-Richardson, R.; Walker, A.; Denning, D.W.; Richardson, M.D. High-volume culture and quantitative real-time PCR for the detection of Aspergillus in sputum. Clin. Microbiol. Infect. 2020, 26, 935–940.

[73] Rahman, H.U.; Yue, X.; Yu, Q.; Zhang, W.; Zhang, Q.; Li, P. Current PCR-based methods for the detection of mycotoxigenic fungi in complex food and feed matrices. World Mycotoxin J. 2020, 13, 139–150.

[74] Kim, D.M.; Chung, S.H.; Chun, H.S. Multiplex PCR assay for the detection of aflatoxigenic and non-aflatoxigenic fungi in meju, a Korean fermented soybean food starter. Food Microbiol. 2011, 28, 1402–1408.

[75] Rahman, H.U.; Yue, X.; Ren, X.; Zhang, W.; Zhang, Q.; Li, P. Multiplex PCR assay to detect Aspergillus, Penicillium and Fusarium species simultaneously. Food Addit. Contam. Part A 2020, 37, 1939–1950.

[76] Tannous, J.; Atoui, A.; El Khoury, A.; Kantar, S.; Chdid, N.; Oswald, I.P.; Puel, O.; Lteif, R. Development of a real-time PCR assay for Penicillium expansum quantification and patulin estimation in apples. Food Microbiol. 2015, 50, 28–37.

[77] Ferrara, M.; Perrone, G.; Gallo, A.; Epifani, F.; Visconti, A.; Susca, A. Development of loop-mediated isothermal amplification (LAMP) assay for the rapid detection of Penicillium nordicum in dry-cured meat products. Int. J. Food Microbiol. 2015, 202, 42–47.

[78] Laforgue, R.; Guérin, L.; Pernelle, J.; Monnet, C.; Dupont, J.; Bouix, M. Evaluation of PCR-DGGE methodology to monitor fungal communities on grapes. J. Appl. Microbiol. 2009, 107, 1208–1218.

[79] Coudray-Meunier, C.; Fraisse, A.; Martin-Latil, S.; Guillier, L.; Perelle, S. Discrimination of infectious hepatitis A virus and rotavirus by combining dyes and surfactants with RT-qPCR. BMC Microbiol. 2013, 13, 216.

[80] La Bella, G.; Martella, V.; Basanisi, M.; Nobili, G.; Terio, V.; La Salandra, G. Food-borne viruses in shellfish: Investigation on norovirus and HAV presence in Apulia (SE Italy). Food Environ. Virol. 2017, 9, 179–186.

[81] Althof, N.; Trojnar, E.; Böhm, T.; Burkhardt, S.; Carl, A.; Contzen, M.; Kilwinski, J.; Mergemeier, S.; Moor, D.; Mäde, D. Interlaboratory validation of a method for hepatitis E virus RNA detection in meat and meat products. Food Environ. Virol. 2019, 11, 1–8.

[82] Szabo, K.; Trojnar, E.; Anheyer-Behmenburg, H.; Binder, A.; Schotte, U.; Ellerbroek, L.; Klein, G.; Johne, R. Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. Int. J. Food Microbiol. 2015, 215, 149–156.

[83] Soares, V.M.; Dos Santos, E.A.R.; Tadielo, L.E.; Cerqueira-Cézar, C.K.; Sampaio, A.N.d.C.E.; Eisen, A.K.A.; de Oliveira, K.G.; Padilha, M.B.; de Moraes Guerra, M.E.; Gasparetto, R. Detection of adenovirus,

rotavirus, and hepatitis E virus in meat cuts marketed in Uruguaiana, Rio Grande do Sul, Brazil. One Health 2022, 14, 100377.

[84] Fraisse, A.; Coudray-Meunier, C.; Martin-Latil, S.; Hennechart-Collette, C.; Delannoy, S.; Fach, P.; Perelle, S. Digital RT-PCR method for hepatitis A virus and norovirus quantification in soft berries. Int. J. Food Microbiol. 2017, 243, 36–45.

[85] Coudray-Meunier, C.; Fraisse, A.; Martin-Latil, S.; Guillier, L.; Delannoy, S.; Fach, P.; Perelle, S. A comparative study of digital RT-PCR and RT-qPCR for quantification of Hepatitis A virus and Norovirus in lettuce and water samples. Int. J. Food Microbiol. 2015, 201, 17–26.

التقدمات المبتكرة في تقنيات تفاعل البوليميراز المتسلسل (PCR) لتحسين الكشف والتعرف على مسببات الأمراض المنقولة عبر الغذاء: مراجعة شاملة

الملخص

الخلفية :تشكل مسببات الأمراض الميكروبية تهديدات كبيرة لسلامة الغذاء والصحة العامة، حيث تؤثر الأمراض المنقولة عبر الغذاء على ملايين الأشخاص حول العالم. غالبًا ما تفتقر طرق الكشف التقليدية إلى الحساسية والسرعة المطلوبة للرصد الفعال. لقد أحدثت التقدمات الأخيرة في تقنيات تفاعل البوليميراز المتسلسل (PCR) ثورة في الكشف عن مسببات الأمراض، حيث توفر تحديدًا سريعًا ودقيقًا للميكروبات المنقولة عبر الغذاء.

الطرق :تقوم هذه المراجعة بتلخيص التطورات الأخيرة في منهجياتPCR ، بما في ذلك PCR المتعدد، و PCRالزمني الحقيقي، و PCRالعكسي (RT-PCR)، وتسلسل الجيل التالي (NGS). تم إجراء تقييم شامل لهذه التقنيات، مع التركيز على تطبيقاتها في تحديد مسببات الأمراض البكتيرية والفطرية والفيروسية عبر مصفوفات غذائية متنوعة. تناقش المراجعة أيضًا التحديات المرتبطة بالطرق التقليدية المعتمدة على الثقافة وتسلط الضوء على فوائد التقنيات الجزيئية الحديثة.

النتائج :تشير النتائج إلى أن طرق PCR تعزز بشكل كبير من الكشف والتقدير الكمي لمسببات الأمراض المنقولة عبر الغذاء مقارنةً بالتقنيات التقليدية. أظهر PCR الزمني الحقيقي حساسية وخصوصية عالية لمسببات الأمراض مثل السالمونيلا، والليستيريا، والإيشيريشيا كولاي، بينما قدم NGS رؤى حول التركيب الميكروبي لعينات الغذاء، مما يمكن من تحديد مجتمعات مسببات الأمراض المعقدة.

الخاتمة :إن دمج تقنيات PCR المتقدمة في بروتوكولات سلامة الغذاء أمر ضروري للتخفيف من المخاطر المرتبطة بمسببات الأمراض المنقولة عبر الغذاء. لا تعمل هذه المنهجيات على تحسين معدلات الكشف فحسب، بل تسهل أيضًا الاستجابة السريعة للصحة العامة تجاه تفشي الأمراض. يجب أن تركز الأبحاث المستقبلية على تحسين هذه التقنيات للاستخدام الروتيني في مختبرات سلامة الغذاء واستكشاف طرق جديدة للتحكم في مسببات الأمراض.

الكلمات المفتاحية :سلامة الغذاء، كشف مسببات الأمراض، تقنياتPCR ، البيولوجيا الجزيئية، الأمراض المنقولة عبر الغذاء.