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Review article

Conventional and advanced detection techniques of foodborne pathogens: A comprehensive review

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ABSTRACT

Foodborne pathogens are a major public health concern and have a significant economic impact globally. From harvesting to consumption stages, food is generally contaminated by viruses, parasites, and bacteria, which causes foodborne diseases such as hemorrhagic colitis, hemolytic uremic syndrome (HUS), typhoid, acute, gastroenteritis, diarrhea, and thrombotic thrombocytopenic purpura (TTP). Hence, early detection of foodborne pathogenic microbes is essential to ensure a safe food supply and to prevent foodborne diseases. The identification of foodborne pathogens is associated with conventional (e.g., culture-based, biochemical test-based, immunological-based, and nucleic acid-based methods) and advances (e.g., hybridization-based, array-based, spectroscopy-based, and biosensor-based process) techniques. For industrial food applications, detection methods could meet parameters such as accuracy level, efficiency, quickness, specificity, sensitivity, and non-labor intensive. This review provides an overview of conventional and advanced techniques used to detect foodborne pathogens over the years. Therefore, the scientific community, policymakers, and food and agriculture industries can choose an appropriate method for better results.

1. Introduction

Food safety ensures the protection of consumer health from foodborne illnesses. Some significant factors, including microbial, chemical and nutritional change, biological diversity, water activity, climate change, and environmental hygiene, can affect the safety of the food [1]. Among the features, foodborne pathogens are a prime harmful element to spoiling food's desirability for consumption, leading to foodborne disorders [2]. Foodborne diseases are associated with pathogens and lead to serious health problems worldwide. The World Health Organization (WHO) estimates that these pathogens are responsible for 23 million foodborne illnesses and 5000

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deaths in Europe every year [3]. The increasing cost of food disease raises medical costs, productivity losses, and illness-related mortality each year [4].

Pathogens such as bacteria, viruses, fungi, yeast, and parasites are commonly responsible for foodborne diseases. They contaminate the food products (freshly produced and raw foods, including fish, meat, poultry, eggs, and dairy) during growth, harvesting, processing, storage, shipping, and preservation. These pathogens enter the human body through gastrointestinal tract routes and cause many foodborne diseases [5]. *Escherichia coli, Salmonella* spp.*, Clostridium* spp.*, Bacillus* spp.*, Vibrio* spp., *Shigella* spp., *Pseudomonas* spp., *Listeria* spp., *Cyclospora* spp., *Campylobacter* spp., *Staphylococcus* spp., *Klebsiella* spp., and *Acinetobacter* spp. are key bacterial species which cause food-borne diseases in humans by pathogenic effects [6–8]. One of the major pathogens responsible for foodborne diseases, such as thrombotic thrombocytopenic purpura (TTP), hemorrhagic colitis, and hemolytic uremic syndrome (HUS), is *Escherichia coli.* The sources of *E. coli* are unpasteurized milk, undercooked and raw meat, fruits, and vegetables. *E. coli* O157:H7 is one of the strains of *E. coli* that produce the Shiga toxin responsible for human illness [9]. *Salmonella* spp. is found in meat, poultry, eggs, dairy products, seafood, and other products of animals that cause foodborne diseases such as typhoid, enterocolitis, and diarrhea [10]. *Clostridium* spp. has the capability of causing food-borne diseases, especially to infants, pregnant women and their fetuses, older adults, and people with weak immune systems through the consumption of contaminated canned foods, fruits, and salted fish. *Clostridium botulinum* can cause headaches, dizziness, blurred vision, weakness, tingling or numbness of the skin, paralysis, and the nervous system of humans [11]. *Vibrio* spp. is responsible for contaminating water and food that causes abdominal pain, food poisoning, gastroenteritis, and acute dysentery, which leads to dehydration and even death [12]. *Shigella* spp. can spread infection by contaminating the food materials during food handling and preparation, which leads to gastroenteritis and diarrhea [13]. *Campylobacter* is responsible for contaminating raw dairy products, raw or undercooked meat, and poultry which is why it causes human illness [14].

Furthermore*, Listeria monocytogenes* is found in raw and undercooked meats, hot dogs, unpasteurized milk, and dairy products, which leads to a foodborne disease called listeriosis [15]. Also, other bacteria such as *Enterobacter* spp., *Staphylococcus* spp., *Campylobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Citrobacter* spp. cause food-borne diseases [16]. Apart from these, viruses such as adenovirus, norovirus, calicivirus, hepatovirus, enterovirus, and rotavirus are responsible for severe foodborne diseases [17,18]. Adenovirus is found in contaminated food, which causes gastroenteritis in young children [19]. Calicivirus and norovirus cause foodborne outbreaks, including acute gastroenteritis and diarrhea [20]. Hepatoviruses include the hepatitis A virus, hepatitis C virus, and hepatitis E virus, which are resistant to food preservation methods and cause food-borne outbreaks [21]. Enterovirus causes fever, malaise, muscle aches, headaches, diarrhea, and vomiting by consuming contained food [22]. Nevertheless, *Aspergillus flavus*, *Xerophilic penicillia*, *Xerophilic aspergilli*, *Eurotium halophilicum*, *Xeromyces bisporus*, *Chrysosporium*, *Eurotium,* and *Rhizopus* are found in bread, ham, dry salami, jams, salt fish, fruit cakes, dry fruit, dry grain, and confectionery, which lead to foodborne diseases [23]. Additionally, *Ascomycetes*, *Zygosaccharomyces rouxii*, *Zygosaccharomyces bailii*, and *Debaryomyces hansenii* are the most common yeasts found in food during the preparation and storage of fruits, vegetables, dairy products, cereal-based products, meat, poultry, sauces, proteinaceous foods, seafood, and sugar-rich products [24]. Furthermore, some parasites include fish-borne Trematodes, *Ascaris*,

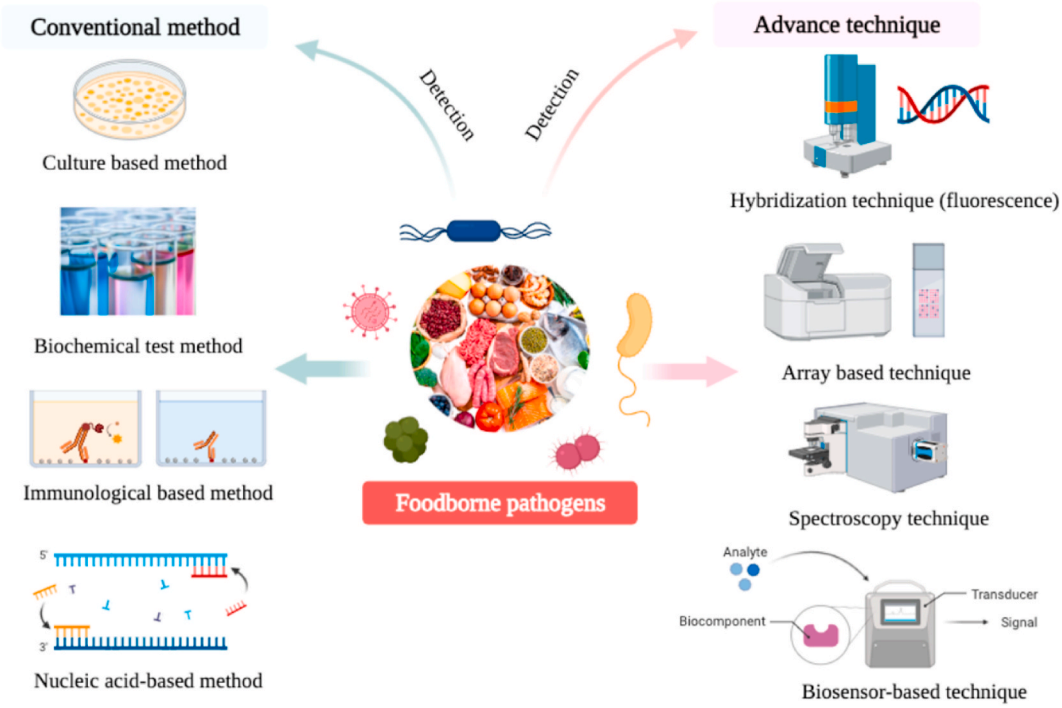

Fig. 1. Conventional and advanced methods for the detection of foodborne pathogens.

Cryptosporidium, *Entamoeba histolytica,* and *Echinococcus* spp. cause food spoilage [25].

Detection of foodborne pathogens is necessary to ensure food safety in the food industry. Research is needed to identify and control the spread of the pathogens before a severe outbreak. The detection method is an integral part of the regulatory compliance of food production and processing. Various detection methods have been developed to identify foodborne pathogens. Classification of detection methods has been divided into different groups according to their principal advantages and disadvantages [26]. This review aims to provide knowledge of the conventional and advanced detection methods of foodborne pathogens. There are many conventional methods such as culture-based methods, biochemical methods, nucleic acid-based methods (polymerase chain reaction (PCR), multiplex PCR, real-time PCR, quantitative real-time PCR (qPCR), and reverse transcriptase PCR), immunological-based methods (enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay, immunomagnetic separation assay, and immunofluorescence assay), and ultrasound, technique (Fig. 1) [27–31]. Although conventional methods provide high selectivity and sensitivity, they take a long time and are labor-intensive. Numerous advanced methods, such as biosensor-based methods and nucleic acid sequence-based detection techniques (DNA microarray, DNA hybridization, spectroscopic technique, instrumental-based methods, aptamers-based method, loop-mediated isothermal amplification (LAMP) based method, and metagenomic assay) have been devolved into the platform for detection and identification of foodborne pathogens [28,31–34]. The list of foodborne pathogens' detection methods is presented in Table 1.

2. Conventional methods

2.1. Culture-based method

The culture-based method is the oldest technique that confirms the presence of foodborne pathogens in contaminated foods. It is a sequential cultural enrichment method consisting of selective and differential plating, confirmation, and strain typing [35]. It can be divided into two categories: pre-enrichment, which revitalizes injured cells, increases the concentration of the target pathogen in the food samples, and rehydrates cells from dried foods. Another is selective enrichment, which uses specific media to improve the amount of a particular pathogen in food samples [28]. The culture condition depends on various nutrients in the media, incubation time, temperature, and atmosphere [36]. A list of pathogens identified using different media is presented in Table 2.

The culture-based method is the fundamental method for developing any alternative method for detecting foodborne pathogens in food samples that is cost-effective [37]. The detection of foodborne pathogens in culture-based ways is selective and distinctive, which extinguishes the growth of unnecessary microbes, and a differential medium is used to identify targeted pathogenic microbes. Rogosa medium is a particular medium used to differentiate the genus *Lactobacillus* and non-lactic bacteria, where sodium acetate, acetic acid,

Table 1
Foodborne pathogens detection methods.

Pathogen	Source	Detection method		References
		Conventional	Advance	
E. coli O157:H7	Unpasteurized milk, raw meat, fruits, and vegetables	Lateral flow immunoassay, ELISA, multiplex PCR, and real time PCR	Electrochemical biosensor, LAMP, and aptamers-based method	[95, 229–234]
Salmonella spp.	Meat, poultry, egg, dairy products, and seafood	Immunomagnetic separation assay, real-time PCR, and multiplex PCR	Biosensor (optical, electrochemical, piezoelectric), aptamers-based method, and LAMP	[140,216, 235–237]
Clostridium spp.	Milk, canned foods, fruits, and salted fish	Culture based method, immunofluorescence assay, ELISA, and real-time PCR	Electrochemical biosensors and LAMP	[193, 238–242]
Vibrio spp.	Dairy product, raw and semi-cooked meat, and lightly salted seafoods	Quantitative real time PCR, multiplex PCR, and ELISA	LAMP and DNA hybridization	[243–247]
Shigella spp.	Milk, blue cheese, meat, and seafoods	ELISA, real time PCR, and multiplex PCR,	Aptamers based method and nanomaterial-based biosensors	[138, 248–251]
Bacillus spp.	Meat, eggs, fruits vegetables, milk, and dairy products	Multiplex PCR, real-time PCR, and lateral flow immunoassay	Silicon-based biosensor	[252–255]
Campylobacter spp.	Dairy product, raw meat, and poultry	Immunomagnetic separation methods and real-time PCR	DNA hybridization and Nanoplasmonic sensor	[14, 256–258]
Adenovirus	Vegetables and fruits	Quantitative real time PCR and reverse transcriptase PCR	LAMP and biosensor	[92,143,259, 260]
Norovirus	Fruits, vegetables, meat, poultry, and dairy product	Immunoassay based method, quantitative real time PCR, and reverse transcriptase PCR	Aptamers based method and photoelectrochemical biosensor	[92, 261–263]
Hepatovirus	Poultry, meat, and meat products	Reverse transcriptase PCR and ELISA	Biosensor and LAMP	[102, 264–266]
Aspergillus spp.	bread, jams, fruit cake, dried fruit, and grain	Polymerase chain reaction	Electrochemical biosensor	[267,268]
Ascomycetes spp.	–	Polymerase chain reaction	Biosensor	[269,270]

PCR: polymerase chain reaction, ELISA: enzyme-linked immunosorbent assay, LAMP: Loop-mediated isothermal amplification.

Table 2
Identification of foodborne pathogen using different selective media along with incubation period and temperature.

Type of pathogen	Source	Type of medium	Incubation temperature/Time	References
E. coli	Meat, dairy product, fruit, and vegetables	Tryptic soy broth (TSB), brain-heart-infusion (BHI), eosin methylene blue agar (EMB), and xylose lysine dextrose agar (XLD).	35–37 °C/18–48 h	[271]
Vibrio spp.	Vegetables, dairy products, raw and dried fish, and seafoods	Tryptic soy broth (TSB), marine broth (MB), blood agar, thiosulfate citrate bile salts sucrose (TCBS) agar, and CHROMagar Vibrio (CV)	37 °C/24 h	[272]
Salmonella spp.	Meat, poultry, egg, dairy products, and seafood	xylose lysine desoxycholate agar, Hektoen enteric agar and bismuth sulfite agar, Salmonella Shigella agar, and McConkey agar,	37 °C/24 h	[273]
Clostridium spp.	Canned food, dried, and liquid food	Prototype chromogenic (IDCd) medium, CLO medium, cycloserine-cefoxitin-fructose-egg yolk agar (CCFA), oxoid medium, and clostridium difficile agar	37 °C/24 h	[274]
Bacillus spp.	Meat, vegetables, and dairy products	Mannitol egg yolk polymyxin (MYP) medium and polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA), and chromogenic agar	37 °C/4–18 h	[275]
Norovirus	Fruit and vegetables	Dulbecco's modified Eagle's medium (DMEM-F12)	37 °C/10 min	[276]
Hepatovirus	Row and slightly cooked food	SeaKem LE agarose medium	37 °C/24–48 h	[277]
Aspergillus spp.	Bread dried fruit, and grain	Sabouraud Dextrose Broth and tomato paste	25 °C/2 months	[278]

and selective inhibitors like nitrite polymyxin B and actidione extinguish the growth of non-lactic bacteria [38]. The culture-based method shows more direct discrimination based on color. Purple or blue colonies on the Cefsulodin-Irgasan-Novobiocin (CIN) agar confirm the presence of *Yersinia enterocolitica* [39]. The major drawback of this method is that it is time-consuming due to the slower growth of microbes; it may take 18–24 h or several days to culture and is labor intensive. *Renibacterium salmoninarum* requires a 12-weeks or more extended incubation period for growth in the selective kidney disease medium (SKDM) [40].

2.2. Biochemical test method

A biochemical test is a growth-promoting method where compounds are used as signals that indicate the presence of pathogens and inhibit the growth of competing microbes (Table 3). Sometimes it involves a culture method technique, in which the pathogen is incubated in a solid or liquid culture medium [41]. There are a lot of biochemical tests which confirm the presence of particular pathogens in food samples. They are the oxidase test, catalase test, indole production test, methyl red, Voges-Proskauer, triple sugar iron agar, blood agar plates, motility agar, mannitol salt agar, starch hydrolysis test, galactose hydrolysis test, carbohydrate fermentation, citrate utilization, urase test, hydrogen sulfide production test, nitrate reduction test, optochin sensitivity test, and

Table 3
Different types of biochemical tests for the detection of foodborne pathogens.

Biochemical test	Chemical characteristics	Chemical reaction	Medium	Color	References
Oxidase test	Identify cytochrome oxidase enzyme	electron transport chain	Agar	Purple	[279]
Catalase test	Detoxifies hydrogen peroxide into water and oxygen	Bubbles of oxygen	Blood agar	_	[280]
Indole production test	Degradation of amino acid tryptophan and production of indole	Oxidizes tryptophan	tryptophan	Pink/ Cherry red	[281]
Methyl red test	Fermentation of sugar	Oxidizes glucose	methyl red and Voges–Proskauer (MR-VP) broth	Red	[282]
Voges-Proskauer test	Production of nonacidic or neutral end product	Ferment sugars via the butanediol pathway	methyl red and Voges–Proskauer (MR-VP) broth	Red	[282]
Triple sugar iron agar test	Production of H_2S	Cytochrome oxidase activity	Triple sugar iron agar	Yellow	[283]
Blood agar plate test	Production of hemolysins	Hemolysis activity	Blood agar	Colorless	[284]
Mannitol salt agar test	Production of acidic byproducts	Fermenting mannitol	Mannitol salt agar	Yellow	[285]
Starch hydrolysis test	Production of amylose and amylopectin	Starch hydrolysis into sugar	Starch agar	Yellow	[286]
Galactose hydrolysis test	Production of glucose	Galactose hydrolysis	Eosin methylene blue medium	Green	[286]
Carbohydrate fermentation test	Extraction of fatty acids	Fermenting mannitol	Proteose peptone medium	Yellow	[287]
Citrate utilization test	Utilizes citrate–carbon source	Fermenting citrate	Simmon's citrate agar	Blue	[288]
Urease test	Detects the alkaline and forms ammonia	Hydrolyzing urea	Christensen's urea agar	Pink	[289]
Hydrogen sulfide production test	Production of hydrogen sulfide	Reducing sulfur compound	Tryptone soya agar	Black	[283]
Nitrate reduction test	Formation of nitrate and ammonium	Reduction of nitrate	Nitrate broth	Red	[290]

bacitracin sensitivity test [31]. Lactic acid bacteria (LAB) isolated from fish and prawns can be identified by producing different enzymes, acids, ammonia, indole, and gas [42].

Volatile compounds such as alcohols, fatty acids, ketones, hydrocarbons, and aromatic compounds are used to detect pathogens. Volatile compound analysis can be directly applied to identifying pathogens in food samples [43]. In addition, volatile compounds such as ethanol and acetic acid can identify *Saccharomyces* spp., *E. coli,* and *Aspergillus* spp. in canned tomatoes [44]. For the extraction and concentration of volatile compounds, methods like solid phase microextraction (SPME), gas chromatography, and mass spectrometry are used [45].

2.3. Immunological-based method

The immunological-based detection of foodborne pathogens is antibody-antigen interactions, where a particular antigen binds to a specific antibody (monoclonal or polyclonal antibody). Monoclonal antibodies are more suitable than polyclonal antibodies for the selective detection of pathogens due to their specificity, sensitivity, reproducibility, and reliability [46]. The sensitivity and specificity of this method are based on the epitope position of the antibody that binds to a specific antigen. It is a rapid and robust method compared to the culture-based method to detect pathogens and their toxins (i.e., mycotoxin) [47,48]. It detects the pathogen's protein toxin and metabolic substances like protein, glycoprotein, and polysaccharides related to the pathogen growth [49]. This assay is expensive, requires pre-enrichment steps, and cross-reactivity with other pathogen antigens may cause false positive results and cannot detect the damaged pathogen's cell [48]. There are a variety of immunological methods which are used for the detection of the pathogen in food (Table 4). They are the enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay, immunofluorescence assay, immunomagnetic separation, latex agglutination, immunodiffusion assays, immunochromatographic assay, immunoglobulin G (IgG) assay, and gold-labeled immunosorbent assay (GLISA).

2.3.1. Enzyme-linked immunosorbent assay (ELISA)

ELISA is an immunological technique that detects antigen-antibody complexes in samples using an enzymatic reaction's sensitivity. This assay uses a microtiter as a solid support, membranes, paddles, dipsticks, pipet tips, and solid matrices [50]. Peroxidase, β-galactosidase, and alkaline phosphatase are the most commonly used enzymes in ELISA [51]. This assay involves attaching the samples covalently to antigen-coated microtiter plates in an alkaline buffer system, using antibodies against pathogen antigens with high affinity, and detecting fluorescent antigen/antibody complexes based on ultraviolet light. A specific protein or toxicant binds to the respective antigen in this assay. This assay generates colors that can be seen with the naked eye, and an ELISA reader measures the wavelength of the colors. The quantitative determination of pathogen numbers in food samples is counted by the number of absorbances, which is proportional to the number of pathogens [52,53]. The advantages of the ELISA system are that it is rapid, specific, less labor-intensive, and capable of using a large number of samples to detect pathogens from a food sample. Antigen variability, the sensitivity limit, and difficulty in measuring enzyme activity are some disadvantages of this method [31]. This assay requires the minimum sensitivity of a traditional medium to produce cell surface antigens and detect them [54]. There are various ELISAs, including direct, indirect, sandwich, and competitive ELISA.

2.3.1.1. Direct ELISA. ELISA is a method where isolated and purified samples (analyte) attach to a solid surface well and then introduce enzyme-labeled polyclonal antibodies to the assay. Pathogens in jarred baby food can be detected using the antibody MVK31 (IgG) developed against the semi-carbazide [55].

2.3.1.2. Indirect ELISA. Indirect ELISA is a method that requires a single antibody directed against a specific antigen that has been immobilized on a surface. The target antibodies bind to coated antigens, and unbound antibodies are washed off in this assay. Also, peroxidase enzyme, anti-immunoglobulin, and substrate buffer are used in indirect ELISA. The substrate buffers produce color. The wavelength of the color is measured by a spectrophotometer, which is proportional to the level of antibodies present in the sample

Table 4
Different immunological-based methods for the identification of various foodborne pathogens in food samples.

Assay name	Pathogen	Food sample	Detection time	Detection limit	References
ELISA	*Escherichia coli* O157: H7	Vegetables	3 h	1×10^4 CFU/mL	[59]
	Salmonella typhimurium	Milk	10 min	1.25×10^6 CFU/mL	[291]
	Cronobacter sakazakii	Fruits	4 h	1×10^4 CFU/mL	[292]
Lateral flow immunoassay	*Listeria monocytogenes*	Raw food	20 min	1×10^4 CFU/mL	[293]
	Shigella boydii	Bread, milk, jelly	5–10 min	1×10^6 CFU/mL	[294]
Immunofluorescence Assay	*Escherichia coli* O157: H7	Meat	1 h	1.2×10^3 CFU/mL	[295]
Immunomagnetic Separation	*Salmonella* spp.	Poultry	2 h	1×10^2 CFU/mL	[296]
Latex agglutination assay	*Campylobacter* spp.	Milk, poultry, and meat	48 h	2.18 to 10.17 log10 CFU/mL	[297]
Immunodiffusion assays	*Salmonella* spp.	Dairy product	14 h	1×10^5 CFU/mL	[69]
Immunochromatographic assay	*Vibrio parahaemolyticus*	Sea food	4.5 h	1.58×10^2 CFU/mL	[298]
Immunoglobulin G assay	*Staphylococcus aureus*	Meat	70 min	3.1×10^2 CFU/mL	[71]
GLISA	*Bacillus cereus*	Spices and herbs	NA	$1.6–2.0 \times 10^3$ CFU/mL	[72]

ELISA: enzyme-linked immunosorbent assay, GLISA: gold labeled immunosorbent assay.

Table 5
Different types of nucleic acid-based method approaches are used for the detection of food-borne pathogens.

Types of nucleic acid-based method	Pathogen name	Target site	Primer	Amplified product size (Base pair)	Probe	References
PCR	*Escherichia coli*	*stx2b* gene	Forward TATACGATGACACCGGAAGAAG Reverse CCTGCGATTCAGAAAAGCAGC	300	NA	[299]
	Salmonella typhimurium	*MDH* gene	Forward CGCATTCCACCACGCCCTTC Reverse TGCCAACGGAAGTTGAAGTG	261	NA	[300]
	Vibrio parahaemolyticus	*VP1332* gene	Forward GTGCGTTGGGATTTGGTCT Reverse GGCGGTAAACTTCGTCAGG	352	NA	[301]
	Aspergillus flavus	Aflatoxin gene	Forward GTAGGGTTCCTAGCGAGCC Reverse GGAAAAAGATTGATTTGCG TTC	500	NA	[302]
Nested PCR	*Yersinia enterocolitica*	*yadA* gene	1st set primer Forward TAAGATCAGTGTCTCTGCGGCA Reverse TAGTTATTTGCGATCCCTAGCAC 2nd set primer Forward GCGTTGTTCTCATCTCCATATGC Reverse GGCTTTCATGACCATGGATACAC	747 529	NA	[303]
	Salmonella enterica	*fliC* gene and *stn* gene	1st set primer Forward GGTCAAAATCCAGCGGTTTA Reverse TTGCTGCTAACGGCGAGA 2nd set primer Forward GCCGGCTTTCAACGCCTCTAC Reverse GACCAAAGCTGACGGGACAG	1318 450	NA	[304]
Real-time PCR	*E. coli* O157:H7	*stx2* gene	Forward ATTAACCACACCCCACCG Reverse GTCATGGAAACCGTTGTCAC	115	SensiFAST Probe	[305]
	Yersinia enterocolitica	*ail* gene	Forward ATGATAACTGGGGAGTAATAGGTTCG Reverse CCCAGTAATCCATAAAGGCTAACATAT		ail probe, Yc probe	[306]
	Campylobacter jejuni	*hipO* gene	Forward CAAAAAAATCCAAAATCCTCACTTG Reverse TGCACCAGTGACTATGAATAACGA	132	6-FAM and BHQ1 probe	[257]
	Bacillus cereus	*ces* gene	Forward CACGCCGAAAGTGATTATACCAA Reverse CACGATAAAACCACTGAGATAGTG	176	SYBR green I	[307]
Quantitative real time PCR	*Escherichia coli*	*uidA* gene	Forward CGGAAGCAACGCGTAAACTC Reverse TGAGCGTCGCAGAACATTACA	1300	Taq Man probe	[308]
	Listeria monocytogenes	*hlyA* gene	Forward GCAACAAACTGAAGCAAAGGAT Reverse CGATTGGCGTCTTAGGACTTGC	2000	*hly* specific probe	[309]
	Salmonella enterica	*ssaN* gene	Forward GCCAGAAGCGTGCTTTTCC Reverse GGGCAACGAGTGGGTATTTTT	–	IAC probe	[310]
Reverse transcriptase PCR	*Vibrio* spp.	*16S rDNA*	Forward ′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGGGCGTAAAGCGCATGCAGGT Reverse GAATTCTACCCCCCTCTACAG	120	NA	[311]
	Escherichia coli	*Shiga toxin(slt*-II) operon	Forward TTAAATGGGTACTGTCCT Reverse CAGAGTGGTATAACTGCTGTC	401	NA	[312]
	Hepatitis A virus	capsid protein-coding region (VP1)	Forward CTCCAGAATCATCTCAAC Reverse CAGCACATCAGAAAGGTGAG	192	NA	[313]
Real-time reverse transcription PCR	Yeasts and molds	*act* gene	Forward CTGGGAYGAYATGGARAAGA Reverse GYTCRGCCAGGATCTTCAT	353	SYBR Green I	[314]
	Salmonella enterica	*invA* gene	Forward CACGCTCTTTCGTCTGGCA Reverse	154	SYBR Green I	[315]

(*continued on next page*)

Table 5 (*continued*)

Types of nucleic acid-based method	Pathogen name	Target site	Primer	Amplified product size (Base pair)	Probe	References
	Listeria monocytogenes	*hly* gene	Forward ACTTCGGCGCAATCAGTGA Reverse TTGCAACTGCTCTTTAGTAACAGCTT	137	SYBR Green I	[316]
Multiplex PCR	*Clostridium perfringens*	*cpa* gene	Forward GCTAATGTTACTGCCGTTGA Reverse CCTCTGATACATCGTGTAAG	324	NA	[317]
		Cpb gene	Forward GCGAATATGCTGAATCATCTA Reverse GCAGGAACATTAGTATATCTTC	196		
		Etx gene	Forward GCGGTGATATCCATCTATTC Reverse CCACTTACTTGTCCTACTAAC	655		
		iA gene	Forward ACTACTCTCAGACAAGACAG Reverse CTTTCCTTCTATTACTATACG	446		
		cpe gene	Forward GGAGATGGTTGGATATTAGG Reverse GGACCAGCAGTTGTAGATA	233		
		cpb2 gene	Forward AGATTTTAAATATGATCCTAACC Reverse CAATACCCTTCACCAAATACTC	567		
	Salmonella spp.	*invA* gene	Forward TTGTTACGGCTATTTTGACCA Reverse CTGACTGCTACCTTGCTGATG	521	NA	[318]
		sefA gene	Forward GCAGCGGTTACTATTGCAGC Reverse TGTGACAGGGACATTTAGCG	330		
		pefA gene	Forward TTCCATTATTGCACTGGGTG Reverse GGCATCTTTCGCTGTGGCTT	497		
	E. coli O157:H7	*rfbE* gene	Forward GCCACCCCCATTTTCGTTG Reverse TCCTCTCTTTCCTCTGCGGT	601	NA	[319]
	Staphylococcus aureus	*nuc* gene	Forward TACAGGTGACTGCGGGCTTATC Reverse CTTACCGGGCAATACACTCACTA	484		
	Salmonella spp.	*invA* gene	Forward CTTTAGCCAAGCCTTGACGAAC Reverse AAAGGCAATACGCAAAGAGGT	284		
	E. coli O157:H7	*Stx1and stx2* gene	Forward GAGCGAAATAATTTATATGTG Reverse TGATGATGGCAATTCAGTAT	518	NA	[95]
	Salmonella spp.	*invA* gene	Forward ACAGTGCTCGTTTACGACCTGAAT Reverse AGACGACTGGTACTGATCGATAAT	284		
	Listeria monocytogenes	*hlyA* gene	Forward CGCAACAAACTGAAGCAAAGG Reverse TTGGCGGCACATTTGTCAC	404		
	Bacterial DNA	*16S rRNA* gene	Forward GTATTGAAAGCTCTGGCGG Reverse TCGCTTAGTCTCTGAACCC	654		

PCR: polymerase chain reaction, NA: not applicable.

[56]. This assay approach detects *Salmonella enterica* in food samples, especially dairy products, eggs, chicken, and meat, based on the monoclonal antibody 4E6F11, which binds to specific antigens [57].

2.3.1.3. Sandwich ELISA. Sandwich enzyme-linked immunosorbent assay is a technique that uses two antibodies against a single antigen. The primary antibody is applied to interact with the target molecule, and the secondary antibody and an enzyme specify the target molecule, producing a sandwich like a primary antibody, target molecule, and secondary antibody complex. The color formation of the target molecule with the enzyme-substrate confirms the presence of pathogens in the food sample. It is more sensitive and specific than other ELISAs [58]. This assay helps to detect *Bacillus cereus, B. thuringiensis, B. licheniformis, B. subtilis, B. perfringens,* and *Staphylococcus aureus* in food samples including rice, meat, eggs, fruits, and vegetables [59,60].

2.3.1.4. Competitive ELISA. The competitive enzyme-linked immunosorbent assay is based on three main elements: a particular antibody that binds to a target antigen, a competing antigen that competes for the binding to the specific antibody, and a signal transducer element that quantifies signal output. This assay is performed in two sets. The first set assay follows the indirect ELISA protocol that produces high nonspecific binding, and in the second step, competing antigens with antibodies that employ signal [61]. It is mainly used as an analytical tool for detecting food pathogens because of its low cost, high throughput, straightforward readouts, and high specificity. It can be used to identify *E. coli* O157:H7 in milk [62].

2.3.2. Lateral flow immunoassay

The lateral flow immunoassay is a visual detection based on the reaction of antibodies and pathogens. In this assay, antibodies are attached to nitrocellulose membranes, and sample slots are placed on gold particles or colloidal latex with antibodies. Then targets laterally move toward bound antibodies by the capillary reaction [63]. This reaction produces two capture zones for the moving sample-one is the target pathogen, and the other is the unbound antibody. The pathogen and antibody reaction has two visual signals: positive and negative response, where the presence of a pathogen is confirmed by the positive signal [64]. This method is efficient because of its simplicity, rapidity, stability, sensitivity, and portability [65]. This assay detects pathogens by fluorescent antibody where a fluorescent reporter molecule (fluorescein isothiocyanate) is labeled with an antibody. It is a direct and rapid method of detecting pathogens from food samples [63,66].

2.3.3. Immunomagnetic separation

Immunomagnetic separation is an assay where antibodies are bound with functionalized magnetic beads to separate and identify pathogens from food samples. This assay is rapid and helpful for large quantities of samples [67]. In this assay, antibodies are coated with magnetic beads for incubation in a refrigerator. After incubation, unbound antibodies are washed away, and then coated antigen-magnetic beads are introduced to a semi-liquid food sample containing pathogen antigen (the whole cell or the pathogen's toxin). This mixture is allowed to incubate for the reaction of antigen and antibody-coated beads [68].

2.3.4. Latex agglutination assay

This assay is an immunological method that determines the pathogen as an antigen from a food sample. The antigen is mixed with latex bead-coated antibody, and visual agglutination of latex beads indicates the presence of the target pathogen in a few minutes. This assay is specific, reliable, and uncomplicated to detect pathogens from food samples [49].

2.3.5. Immunodiffusion assays

This assay detects antigens where antigens and antibodies move freely in the diffusion support medium. Firstly, the food sample is inoculated in a semisolid diffusion medium where antibodies are added to the distal surface of the medium. Pathogens move out of the sample and are immobilized by utilizing antigen-antibody complexes. After 14 h, this complex will produce an immunodiffusion band [69].

2.3.6. Immunochromatographic assay

The immunochromatography assay is a simple method intended to detect the presence or absence of the protein serum of a pathogen. It is a dipstick assay that contains pre-enrich selective media. Pathogens from food samples are captured on the dipstick [70].

2.3.7. Immunoglobulin G assay

Immunoglobulin G is a useful assay for targeting virulence in clinical microbiology. Also, this method has been applied to detect foodborne pathogens such as *Campylobacter jejuni* [29]. The advantage of this method is that it can easily detect pathogens using the precipitation technique [71].

2.3.8. Gold-labeled immunosorbent assay

This assay was developed to rapidly detect pathogen toxin (enterotoxin) from food samples (dried dietary foods) with a low detection limit. It is commercially available as the GLISA kit [72].

2.4. Nucleic acid-based method

Nucleic acid amplification is a molecular method based on identifying and quantifying specific pathogen genomes (DNA/RNA). It is a better method than any other conventional detection method for food pathogens because of its rapidity, sensitivity, accuracy, specificity, robustness, minimized risk of contamination, and capacity to identify small amounts of target nucleic acid in food samples [73]. This method's principle amplifies various pathogen's genomes where specific primer sets are used for replication by following three desired range of temperature steps: denaturation, annealing, and extension, where buffer systems in different thermos-cycler machines are used. Agarose gel electrophoresis and ethidium bromide staining detect nucleic acid products [74].

The first step in the nucleic acid-based detection method is selecting the specific sequences used to detect pathogens. A conserved gene with specified sequences is used to choose a target sequence of the bacteria [75]. Ribosomal sequences are widely used as target sequences for detection development, which contains a bacterial ribosomal RNA (rRNA) operon, a 16S rRNA, a 23S rRNA gene, and an intergenic spacer (IGS) region. Generally, the 16S rRNA gene is targeted in the molecular bacterial detection [76,77]. The target sequence for virus detection is the coat protein gene, DNA polymerase gene, or RNA polymerase gene [78]. Sometimes RNA viruses are prone to mutation and have no conserved sequences, which is challenging in detecting food-borne pathogens [79].

A nucleic acid-based method requires timely monitoring and effective surveillance. There are some difficulties in the detection method because the food particles inhibit the technique, and it cannot differentiate the DNA from live cells or dead cells. To solve these difficulties in, the nucleic acid-based method needs to be developed: including purification, cell lysis, nucleic acid extraction, failed reaction, cross-contamination, non-target cell DNA competing, and false positive signal. It is essential to make a well-designed primer that does not amplify the non-target sequences [80]. There are various types of nucleic acid amplification-based methods-polymerase chain reaction (PCR), nested PCR, real-time PCR, quantitative real-time PCR (qPCR), reverse transcriptase PCR (RT-PCR), real-time reverse transcription PCR (Rti-RT-PCR), and multiplex PCR [33]. The identification of pathogens using different nucleic acid-based methods is given in Table 5.

2.4.1. Polymerase chain reaction

Polymerase chain reaction (PCR) is a detection method that amplifies a specific DNA fragment by performing three cyclic steps: (1) denaturing the target DNA at a high temperature (90–95 °C), (2) annealing the two oligonucleotides (primer) at opposite strands of the target DNA at a specific low temperature (55–60 °C), and (3) polymerization of the oligonucleotides that are complementary to the target DNA at an intermediate temperature (70–72 °C) [81,82].

2.4.2. Nested PCR

Nested PCR is required in high-sensitivity situations to eliminate false-positive incidents. It is a combination of two PCR methods: firstly, amplification of target DNA fragment from a sample by the first set of primers, and finally, the second set of primers is used to sequence at the 3′ side of the first primer, which corrects the first round of PCR product [83,84].

2.4.3. Real-time PCR (RT- PCR)

It is a rapid pathogen detection method that correlates PCR product concentration to fluorescence intensity. The fluorescence signal is proportional to the PCR product amount [85]. It is simple to record the fluorescence emission of each cycle compared to the conventional PCR method. There are two fluorescent chemicals to detect and quantify the presence of pathogens in food:fluorescent probes that bind specific DNA sequences (i.e., TaqMan probes) [86] and fluorescent dye which intercalates into DNA (i.e., SYBR Green) [87]. It reduces cycle times by using fluorogenic labels but cannot monitor the size of the amplified product and sometimes has restrictions on the fluorogenic chemistry [88]. However, nonspecific binding and overlapping emission spectra are the main drawbacks of this method.

2.4.4. Quantitative real-time PCR (RT qPCR)

It is an advanced PCR-based reaction that amplifies and quantifies to detect the target DNA. This method uses fluorescent dyes and DNA probes to measure the amplified products [89]. It is a rapid and reliable method because there is no need for post-treatment, reducing the effort and time to detect pathogens [90].

2.4.5. Reverse transcriptase PCR

It is a modified conventional PCR method for RNA transcripts. Firstly, mRNA molecules are converted into single-stranded complementary DNA (cDNA) by the reverse transcriptase enzyme, and next, single-stranded cDNA molecules are converted into double-stranded DNA molecules. Then, these double-stranded DNA molecules can be used as templates for the conventional PCR method [91]. Reverse transcriptase PCR is best for detecting enteric RNA viruses (i.e., Norovirus and HAV) [92].

2.4.6. Real-time reverse transcription PCR (Rti-RT-PCR)

This method was developed to detect pathogens using specific primers and probes combined with reverse transcription PCR. It is more sensitive and rapid than the conventional reverse transcription PCR [93]. It detects RNA viruses like norovirus, astrovirus, and sapovirus [94].

2.4.7. Multiplex PCR

This method consists of two or more primer sets used in a single reaction to produce multiple amplicons specific to various DNA

sequences. It enables amplifying multiple target sequences in a single reaction [95]. This assay can discriminate between actual and false results where some primers are targeted to detect the presence of pathogens, and other primers are used to target the interesting sequence [96]. The primers used in amplification should be a distinct size designed for separate amplification, which depends on the essential components-deoxyribonucleoside triphosphates (dNTPs), enzyme activity, $MgCl_2$ concentration, and target DNA nature [95]. The potentiality of this method is to save time, cost, and effort in identifying bacteria, viruses, and parasites. Still, it poses some difficulties in sensitivity, specificity, optimization process, and preferential amplification of specific targets [97].

3. Advanced method

3.1. Hybridization-based method

The hybridization-based method is an advanced molecular method based on complementary DNA and RNA sequences to detect the presence of specific genes of pathogens. Synthetic complementary DNA fragments (single or double-stranded), called probes, are labeled with fluorescent dyes and used to target the nucleic acid of specific pathogens [98]. It is a rapid, stable, and sensitive fluorescence-based detection method to detect the pathogen by the signal of its presence in the amplification [99]. Hybridization can be used in the assay of fluorometric [100], colorimetric [101], electrochemical [102], and chemiluminescent [103]. This method produces two hairpins through a cascade reaction to form double helices of long-nicked DNA with the probes. The modified hairpin probes generate signals while binding with the hybridized product to indicate the presence of pathogens [104].

Listeria monocytogenes can be detected in ready-made food by two probes- MNP_{250}-$probe_1$ and MNP_{30}-$probe_2$ based on a magnetic DNA-based hybridization reaction with a detection limit of 50 CFU/mL in 2 h [105]. Detection of *Salmonella* spp. is based on Fe_3O_4 magnetic bead with the two carboxyfluorescein (FAM) labeled DNA hairpin probes such as H1-FAM and H2-FAM in spiked lettuce with a detection limit of 6.9×10^2 CFU/g [99]. *E. coli O157:H7* is hybridized between target single-strand DNA and aptamer with the probes with a detection limit of 8.35×10^2 CFU/mL from milk [106].

3.2. Array-based method

An array technique detects the pathogen by DNA sequences, RNA transcripts, and proteins by in situ and ex-situ synthesis of biomolecules on the solid substrate surface. It identifies the interaction between the target and probe, which addresses spatial screening of the microarray format [107]. Array technology is essential with the advantages of rapidity, sensitivity, high accuracy, and throughput [108]. DNA-based array (microarray) and alternative array-based technologies are used to develop the detection of pathogens in food samples.

3.2.1. DNA microarray

DNA microarray is a well-established tool for detecting and quantifying pathogens that measure the genes expression level [109]. DNA microarray is an advanced technology where immobilized nucleic acids (oligonucleotides, genomic DNA, and cDNA) are exposed to nucleic acid probes on solid surfaces (i.e., nylon membranes, glass slides, and silicon chips) [110]. It can be used to confirm and characterize microbes in an easy, fast, cost-effective, and reliable way that is better than the polymerase chain reaction [111]. Nucleic acid probes with fluorescent are amplified with test oligonucleotides and hybridized to surface chips with target immobilized single-stranded nucleotides where each chip can hold hundreds of target nucleotides [109]. It allows the determination of multiple species simultaneously, which provides the virulence gene data. There are four chromosomal loci: 16S rRNA, dnaJ, recA, and gyrB are targeted by 853 probes to detect the bacterial and viral genome sequences in shrimp and fish samples. Amplification and labeling of the nucleic acid of pathogens are done by multiplex PCR and the Klenow fragment-based method [112]. However, the confusion of first-time users and non-reproducible results are the main disadvantages of this method. A list of foodborne pathogens assessed by different types of arrays is shown in Table 6.

3.2.2. Alternative array-based detection

The carbohydrate-based array is one of the powerful methods to detect pathogens by the carbohydrate-mediated interaction with

Table 6
Array-based method for pathogens detection.

Pathogen name	Target site	Probe	Array Matrix	Detection limit	References
Escherichia coli	*ssrA* gene	EC-cap-probe THIOL-AAGTTTTAACGCTTCAACCC EC-cap-control THIOL-TTCAAAATTGCGAAGTTGGG	Microfluidic device fabrication	0.2 nM target DNA	[320]
Salmonella spp.	*Sdf* gene	Btn-TG-T10-AATCAGCCTGTTGTCTGCTCACCATTC-30 Btn-TG-T10-AGATCATCGTCGACATGCTCAC-30	DVD chips	0.2 pg genomic DNA	[321]
Campylobacter Jejuni	*hipO* gene	Btn-TG-T10-CATTGCGAGATACTATGCTTTG-30 Btn-TG-T10-CTGTAAGTATTTTGGCAAGTTT-30	DVD chips	0.2 pg genomic DNA	[321]
Listeria monocytogenes	*Iap* gene	5′polyT15-ATACGATAACATCCA CGGCTCTGGCTGG	Polystyrene strip plate	5.0×10^2 CFU/mL	[322]

the proteins of pathogens. The mannose-coated microarray detects *E. coli* and *Salmonella* spp., which shows the allelic variation of pathogens [113]. Pathogens are detected using a lectin-based microarray because glycan interacts with the lipopolysaccharide of bacterial cells. This lectin array provides rapid identification and differentiation of various bacteria by glycosylation [114]. Gram negative bacterial cells such as *Campylobacter jejuni, Escherichia coli, Lactobacillus* spp., and *Pseudomonas* spp. are identified by lectin-based microarray [115].

3.3. Spectroscopy technique

This is an analytical method for interacting electromagnetic radiation and matter for qualitative and quantitative analysis [116]. This technique is used for rapid pathogen identification in food. Several spectroscopic techniques have been used to identify microbial contamination, including Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and hyperspectral imaging [117]. This method is sensitive toward the surface of the molecule. However, time-consuming, and interference with fluorescence are the main demerits of this method.

3.3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR is a technique based on the infrared (IR) spectrum that works in mid-infrared spectroscopy (MIR) (400–4000 cm^{-1}). It is a quick and reasonably accurate biochemical fingerprinting technique for detecting foodborne pathogens, along with multiple statistical approaches [118]. The FTIR spectroscopy technique involves the observation of the vibrations of excited molecules through an infrared light beam, and the resulting absorbance spectrum represents a specific biochemical or chemical substance. It can be used to estimate biochemical changes in food and provides information on the presence of microbial metabolites in the food [119]. This technique is suitable for the rapid detection of bacterial strain typing [120], quality assessment of the meat products [121], and monitoring microbiological spoilage of seafood [122]. *Bacillus cereus, Bacillus cytotoxicus, Bacillus thuringiensis, Bacillus mycoides,* and *Bacillus weihenstephanensis* are detected by the FTIR spectroscopy technique [123].

3.3.2. Raman spectroscopy

Raman spectroscopy is a technique of spectroscopy that uses vibrational, rotational, and low-frequency modes in each system. It takes advantage of the effect that laser light (in the visible, near-infrared, or near ultraviolet range) is elastically scattered into a small range when interacting with the molecules. While this process occurs, the energy transfer occurs between the incident photons and the molecules [124]. The technique is highly suitable for detecting various analytes from food samples due to its ability to highlight the molecular composition of the target sample with specific criteria [125]. Sometimes, it is difficult to identify specific micro-chemical analyses by ordinary Raman spectroscopy. Surface-enhanced Raman scattering (SERS) is a form of Raman spectroscopy that absorbs a sample on a metal surface (e.g., gold or silver) with nanometer roughness, and the Raman signal of the sample molecules will be significantly improved [126]. Detection and classification of *Staphylococcus aureus* and *E. coli* can be done by Raman spectroscopy [127]. Additionally, a potential approach for quickly identifying pathogenic microorganisms such as *Vibrio* spp., *Shigella* spp., and *Listeria* spp. using Raman spectroscopy combined with machine learning enhanced the accuracy range (87–95%) in detecting pathogens [124].

3.3.3. Hyperspectral imaging techniques (HSI)

The hyperspectral imaging technique (HSI) is one of the emerging technologies for tracking food quality in real time. This technique aims to obtain the spectrum for each pixel in the image (from a scene) to identify the analytes [128]. This technique provides rapid and exact determination of food quality by analyzing microbial contamination or spoilage of food in the processing [129]. A hyperspectral reflectance imaging protocol is the available spectral and hyperspectral imaging technique for detecting bacteria. The methods operate in the range of three central regions near-infrared (NIR), mid-infrared (MIR), and far infrared (FIR) spectrum [130]. Traditional spectroscopy and hyperspectral imaging techniques, when compared to modern imaging techniques, can provide spectral and spatial information detecting target samples and can be used as a visual and smart technology for pathogen analyses in food products. However, this technique is quite complex and expensive [131].

3.4. Loop-mediated isothermal amplification (LAMP)

The LAMP is a method for detecting food-borne pathogens that use four sets of primers to identify six distinct zones on the desired gene. This method requires Bst polymerase, deoxynucleotide triphosphates (dNTPs), betaine, magnesium sulfate, and a buffer [132]. It is a more rapid, sensitive, and reliable technique for the detection of pathogens than conventional PCR due to its ability to produce a considerable number of amplicons, which is around 103 times higher than conventional PCR products in a short period (almost 1 h) [80]. Foodborne pathogens such as *E. coli, Vibrio cholera, and Salmonella* spp. can be detected in chicken and meat products within 1 h using the LAMP technique [133].

LAMP can be quantitative by correlating turbidity signals with the number of DNA copies in the sample. This method has been applied to detect multiple bacterial and viral agents such as *E. coli*, *Campylobacter* spp., *Salmonella* spp., *Vibrio* spp., and norovirus in food [134]. The fluorescent DNA-binding dye-based LAMP method helps to detect the amplification and quantify the accurate number of reactions. A visual-mixed-dye (VMD) containing calcein (precombined with $MnCl_2$) and hydroxynaphthol blue (HNB) for LAMP is used for the detection of foodborne pathogens such as *Vibrio* spp. and *Staphylococcus aureus* in food samples [135]. This method has great potential for future application in detecting foodborne pathogens by modifying consumables such as reagents and microchips.

Real-time electrochemical LAMP is a high throughput electrochemical microsystem device that uses microfluidic chips or DNA sensors to differentiate targeted nucleic acid sequences of pathogens. This microdevice is small, stable, portable, and easy to detect pathogens in food. The detection of *E. coli* O157:H7 and *Salmonella* DNA in food samples can be done by rapid real-time LAMP [136].

3.5. Biosensor

A biosensor is an analytical device that is designed to detect an analyte (target pathogen) by biological sensing elements for a specific, measurable signal, and a physical transducer stores, amplifies, manipulates, and analyses the signal [137,138]. The biosensor beaded detection method is easy, low cost, rapid, and highly selective for identifying foodborne pathogens. In contrast, conventional methods have lower detection, take a long time, are labor intensive, less selective, and less specific. It is an ideal detection method for identifying foodborne pathogens compared to conventional methods because conventional methods cannot detect VBNC (Viable but nonculturable) from food [139]. The biological sensing elements that recognize the target foodborne pathogen are called bioreceptors (such as antibodies, enzymes, aptamers, antimicrobial peptides, bacteriophage, biomimetic, cell, tissue, and nucleic acid probe). They are immobilized on the surface of the transducer to interact with selective analyte molecules [140]. A biosensor is established on a supporting matrix where bioreceptors are attached to detect analytes. The most commonly used sensor matrices are paper, carbon paste, graphite, glassy carbon electrode (GCE), indium tin oxide (ITO), and screen-printed electrodes (SPE), which are selected based on the analyte and mechanism of the transducer. A biosensor's capacity depends on the material's nature, design, and sensor matrix field fabrication type [137]. A transducer is a detector element of a biosensor that converts recognition element signals into a measurable form. The signal can be measured either directly or indirectly. Direct detection is the identification of a target by a single ligand (i.e., antibody), and indirect detection is the identification of a target by a double ligand where the primary ligand bounds on the

Table 7
Biosensors for the detection of foodborne pathogens.

Biosensor	Target pathogen	Source	Bioreceptor	Electrode	Detection limit	Assay time	References
Amperometric biosensor	*Escherichia coli* O157:H7	Milk	Antibody	Nickel oxide thin film	10 cells/mL	–	[148]
	Salmonella Typhimurium	Milk	Antibody	Gold screen-printed	10 CFU/mL	125 min	[323]
Voltammetric biosensor	*Listeria monocytogenes*	Dairy products, meat	Enzyme	Gold nanoparticles carbon	9 CFU/mL	1 min	[324]
	Salmonella spp.	Milk	Antibody	Urease coated gold nanoparticles	10^1 CFU/mL	14 min	[325]
Potentiometric biosensor	*Salmonella typhimurium*	Apple juice	Antibody	Paper-based strip	5 cells/mL	1 h	[156]
	Staphylococcus aureus	Pig	Cell	Aptamer based graphene	8×10^2 CFU/mL	–	[326]
Impedimetric biosensor	*Salmonella* spp.	Milk	Cell	Nisin molecules with gold	1.5×10^1 CFU/mL	1 min	[327]
	Escherichia coli O157:H7	Culture	Cell	Fabricated affinity peptides	20 CFU/mL	30 min	[328]
Colorimetric biosensor	*Staphylococcus aureus*	Culture	DNA	Aptamer based	81 CFU/mL	5.5 h	[208]
	Campylobacter jejuni	Chicken, meat	Antibody	Lactoferrin-based	10 CFU/mL	20 min	[329]
Fluorescent biosensor	*Escherichia coli* O157:H7	Apple juice	Protein	Magnetic DNAzyme-copper	1.57 CFU/mL	1.5 h	[175]
	Listeria monocytogenes	Culture	Cell	Aptamer-functionalized upconversion nanoparticles	8 CFU/mL	2 h	[330]
SPR biosensor	*Campylobacter jejuni*	Chicken	Antibody	surface plasmon resonance	131 ± 4 CFU/mL	–	[177]
	Listeria monocytogenes	Milk	Carbohydrate	Wheat germ agglutinin	3.25 log CFU/100	–	[179]
SERS biosensor	*Salmonella typhimurium*	Culture	DNA	Gold modified magnetic nanoparticles	4 CFU/mL	40 min	[331]
	Staphylococcus aureus	Orange juice	Cell	Aptamer-Fe_3O_4@Au magnetic nanoparticles	21.1 ± 1.5 cell/mL	50 min	[135]
Piezoelectric biosensor	*Campylobacter jejuni*	Poultry	Antibody	Magnetic nanobeads	20–30 CFU/mL	30 min	[332]
QCM biosensor	*Escherichia coli* O157:H7	Culture	DNA	Aptamer based quartz crystal microbalance	1.46×10^3 CFU/mL	50 min	[333]
SAW biosensor	*Escherichia coli*	Packaged food	Antibody	Aluminum nitride based	6.54×10^5 CFU/mL	10 min	[191]
Magnetostrictive Biosensor	*Salmonella Typhimurium*	Poultry and meat	Cell	Carbon-coated copper grid	10^8 CFU/mL	25 min	[334]

SPR: Surface plasmon resonance, SERS: Surface-enhanced Raman scattering, QCM: quartz crystal microbalance, SAW: surface acoustic wave, CFU: colony forming unit.

surface of the sensor to capture the analyte and the secondary ligand generates signal [141]. Biosensors are classified into three major categories-electrochemical, optical, and piezoelectric biosensors based on the types of signal transducers. The detection methods of pathogens using biosensors are presented in Table 7.

3.5.1. Electrochemical biosensor

Chemical information is transferred into the analytical signal by an electrochemical biosensor. Bioreceptors are fixed on the electrode surface, which identifies the target sample. Analyte binds to receptors and triggers the electric properties to provide an electrical signal due to chemical reactions (oxidation and reduction reactions) [142,143]. The method measures and analyzes the target's qualitative and quantitative analysis. The advantages of this method are ease of use, rapid response, higher sensitivity, and greater stability [144]. However, the performance is influenced by environmental factors such as pH and temperature. Based on electric parameters, this biosensor can be classified into four categories: amperometric biosensor, voltammetric biosensor, potentiometric biosensor, and impedimetric biosensor [140,145].

3.5.1.1. Amperometric biosensor. An amperometric biosensor measures the current quantity generated by redox reaction at the working electrode at a constant potential. This redox reaction involves the analyte (pathogen), which produces electrons at a continual potential based on the surface electrode. A linear relationship is created, which is proportional to the concentration of the analyte and measures the current [146]. This amperometric-based biosensor consists of three electrode cells (one working electrode, one reference electrode, and one auxiliary electrode), a source of voltage, and a device for quantifying current and voltage [147]. This biosensor detects *E. coli* [148], *Salmonella* spp. [149], and *Bacillus* spp. [150].

3.5.1.2. Voltammetric biosensor. A voltammetric biosensor measures the current caused by the reaction of oxidation and reduction of electrochemically active analytes (pathogens) where the intensity of the peak is proportional to the analyte concentration [146]. This sensor requires a system of electrode electrochemical cells with a potentiostat to detect the measured current. The biosensor's surface should be developed for analyte identification, specific interaction, and to suppress non-specific interaction [151]. The biosensor detects *E. coli* when polyaniline and gold nanoparticles (AuNPs) are used in the surface electrode [152]. The voltammetric biosensor has developed a rolling circle amplification (RCA) strategy to detect pathogens by DNA concentration of pathogens where target DNA is attached to the electrode surface using a DNA-AuNPs probe. The DNA-AuNPs probe recognizes the RCA product after several rounds of amplification and produces an amperotic signal. This modified voltammetric biosensor can detect *Cronobacter sakazakii, Salmonella* spp*., and E. coli.* [142]. This biosensor is highly sensitive to multiple analytes' measurements and random detection. There are various voltammetric techniques: cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry for analyzing foodborne pathogens [143].

3.5.1.3. Potentiometric biosensor. A potentiometric biosensor measures the voltmeter to detect the difference in electrical potential between the working electrode and the reference electrode under zero current flow. It detects pathogens by electrical signals from changing pH, ions, and redox on the surface [153]. This biosensor comprises a working and a reference electrode within an electrochemical cell. Bioactive materials (i.e., enzymes) coat the working electrode. Sometimes, potentiometric biosensors contain ion-sensitive fields and ion-selective electrodes for the transistor effects [154]. Accumulating ions on an ion-selective surface interface produces a signal to measure the low analyte concentration in a food sample [143]. This biosensor is highly sensitive to detecting label-free pathogens such as *E. coli* [155], *Salmonella* spp. [156], and *Staphylococcus aureus* [157].

3.5.1.4. Impedimetric biosensor. An impedimetric biosensor, also called a capacitive biosensor, is one of the earliest methods to detect foodborne pathogens. This biosensor analyses impedimetric signals of pathogens on the sensing surface [158]. The interaction complex produces a voltage signal because of capacitive and inductive effects. Impedimetric biosensors are based on specific bioreceptors that bind to appropriate analytes (pathogens) such as antibodies, nucleic acids, aptamers, and affinity proteins [159]. This biosensor is classified into two classes based on the presence or absence of a bioreceptor: one measures the impedance change due to the interaction of the target and bioreceptor on the electrode surface, and the other produces metabolites due to the growth of pathogens [160]. The advantages of this sensor are a rapid, easy measurement of a pathogen, no requirement for an enzyme, and the capability of monitoring a large number of samples. The surface activity of an impedimetric biosensor developed with AuNPs-modified electrodes assembled with protein G increases. *E. coli* O157:H7 attaches to an AuNPs-modified surface, which produces an impedimetric signal by strong interaction [161].

3.5.2. Optical biosensor

An optical biosensor is a device that detects optical signals (phase, frequency, and amplitude of light) by the interaction between an analyte (pathogen) with the bioreceptor [162]. Bioreceptors recognize and connect the analyte, and the transducer produces a measurable optic signal from molecular interaction. This sensor detection method depends on various signals: absorption, reflection, refraction, infrared, polarization, dispersion, chemiluminescence, fluorescence, and phosphorescence [153]. An optical biosensor consists of a light source, a transmission medium, a biofunctional surface with a bioreceptor, and a visual detection system [163]. The benefits of optical biosensors are rapid, selective, easy integration, sensitivity, reduction of sample transportation, sample volume, and detection of multiple parameters in different wavelengths [164]. For foodborne pathogen detection, optical biosensors are mainly classified into four types: colorimetric biosensors, fluorescent biosensors, surface plasmon resonance (SPR), and surface-enhanced

Raman scattering (SERS) [140].

3.5.2.1. Colorimetric biosensor. A colorimetric biosensor detects a specific analyte by quickly changing color using the naked eye or an optical detector system to measure the number of pathogens. This biosensor has the advantage of rapid response, and easy operation and is free from complicated apparatus [165]. Colorimetric biosensors can be studied into two categories based on the optical features of nanoparticles and enzymes [140]. The AuNP-based colorimetric biosensor assay is the most sensitive and efficient nanoparticle method for identifying foodborne pathogens. A functionalized AuNP probe (AuNP with DNA strand) is used to detect oligonucleotides. The hybridization of the DNA strand causes a pink to purple color change, which confirms the presence of a specific oligonucleotides [166]. This method requires the isolation of DNA from a pathogen which is laborious and time-consuming. An AuNP conjugated with an antibody is used for label-free identification of pathogens where oval-shaped AuNPs in high-salt solutions stabilize an aptamer effect to detect pathogen [167]. It is a simple, rapid, and appropriate method to detect *Salmonella typhimurium and Salmonella enterica* [168], and *Staphylococcus* spp. [169]. Another type of colorimetric biosensor is enzymatic, which is based on enzyme catalytic reactions. The detection of pathogens is based on the enzyme catalytic reaction and growth of AuNPs in an immunoassay. Pathogens are labeled with catalase in H_2O_2 solution with AuNPs, which produces a colorimetric signal [170]. The detection of *Salmonella typhimurium* is based on the colorimetric sensor, which uses the urease enzyme with antibody-modified silver nanoparticles (AgNPs) [171].

3.5.2.2. Fluorescent biosensor. A fluorescence-based biosensor is a device that obtains a signal from the fluorescence phenomenon where fluorophores (fluorescently labeled molecules) absorb electromagnetic radiation to produce photoluminescence. It has the advantages of rapid response, simple operation, easy readout, high sensitivity, reduced background effect, noncontact detection, and the potential for multiple analyses to detect foodborne pathogens [172]. The sensing label of this biosensor is based on organic dyes (fluorescein, rhodamine dyes, sulforhodamine, and cyanine dyes), nanomaterials (gold nanoparticle (AuNP) composite, silica nanoparticles, magnetic nanoparticles, quantum dots (QDs), up-conversion nanoparticles (UCNPs), graphene oxide, up-conversion nanoparticles, carbon dots, and metal-organic frameworks (MOFs) [173]. A fluorescent biosensor is better than conventional fluorescent immunoassays because many fluorescent molecules can bind to antibodies, enzymes, nucleic acids, and whole cells to produce signals of a significant amplification [174]. The detection of *E. coli* O157:H7 by the fluorescent magnetic biosensor has become simple and highly efficient, with the fluorescent sensor producing a triple signal amplification of magnetic beads, photoluminescence, and enzyme [175].

3.5.2.3. Surface plasmon resonance (SPR) biosensor. A surface plasmon resonance (SPR) assay-based biosensor measures the interaction between the bimolecular binding of targets and ligands [176]. This biosensor recognizes and interacts with an analyte (pathogen) SPR transducer, which produces a signal through the biorecognition elements of the sensor. These biorecognition elements are immobilized on the surface of a metal film that supports a surface plasmon that alters the wave of light on the surface plasmon. The characteristics of the light wave, such as coupling wavelength, coupling angle, phase, and intensity, are regulated by surface plasmon, which is measured for detecting pathogens [153]. This physical optic phenomenon is a label-free technique, free from additional assays, reagents, and sample preparation steps [143]. A surface plasmon resonance biosensor can detect *Campylobacter jejuni* in chicken [177], S*almonella typhimurium* in romaine lettuce [178] and *Listeria monocytogenes* in milk [179]. SPR biosensor allows real-time monitoring of specific pathogen binding (i.e., *Salmonella* spp.) [140].

3.5.2.4. Surface-enhanced Raman scattering (SERS) biosensor. Surface-enhanced Raman scattering biosensor combines Raman spectrum and nanotechnology to determine the targets absorbed on the surface of colloidal nanoparticles (i.e., copper, silver, and gold) [180]. It is a reliable tool for detecting pathogens in food because of its low cost, rapid, specific, sensitive, multiplex analysis, simultaneous characterization, and identification of the target [181]. The detection of foodborne pathogens based on SERS is divided into two categories: (1) label-free biosensors (direct) and (2) label-based biosensors (indirect) [138]. The label-free SERS biosensor acquires a direct intrinsic Raman fingerprint where there is no need for fluorescent labels. It requires one recognizing element, which leads to the sample of analysis. This sensor provides the patterns of interactions of molecule and analyte (i.e., antibody-antigen interaction) [182]. This sensor offers real-time quantification of biomolecular reaction products for recording continuous data and allowing kinetic measuring of ligands [135]. The label-based SERS biosensor uses a nanomaterial probe to recognize biological molecules and Raman active dye, producing an output signal [138]. A Fe_3O_4 magnetic AuNPs nanoparticle is used as a probe in label-based SERS biosensors for detecting specific pathogens [135]. Additionally, the SERS sensor integrated with the new covalent organic frameworks based on biologic interference-free Raman tags showed high sensitivity, with the lowest concentration (10^1 CFU/mL) in the detection of *Salmonella enteritidis* and *E. coli* [183]. Moreover, simultaneous and ultrasensitive detection of other bacteria may be accomplished by altering the loading Raman reporters and particular antibodies, offering a universal SERS platform for the simultaneous detection of various bacteria.

3.5.3. Mass-sensitive biosensor

A mass-sensitive biosensor is a biosensor that uses a crystal surface to produce a signal at a particular frequency. In this biosensor, analytes are recognized on the surface of a crystal where a magnitude is produced by a vibrational frequency [184]. This biosensor is equally important as an optical sensor for sensing biochemicals and the biorecognition [185]. Mass-Sensitive biosensors such as piezoelectric and magnetostrictive detect pathogens from food samples.

3.5.3.1. Piezoelectric biosensor. A Piezoelectric biosensor is a system of electrical and mechanical interaction that assimilates the piezoelectric resonator for the transduction of the signals [186]. It is a microbalance of quartz crystal with a thin circular plate with an electrode (gold). The principle of the piezoelectric biosensor is based on the oscillator, impedance, and impedance scanning that drive the circuit of the resonant frequency [187]. This biosensor is developed for direct detection, label-free, and cost-effectiveness where antigen and antibody interaction can be detected by frequency [188]. The quartz crystal microbalance (QCM) biosensor and surface acoustic wave (SAW) biosensor is the most commonly used piezoelectric biosensor to detect foodborne pathogen [154]. The QCM biosensor is based on the principle of a piezoelectric biosensor and measures frequency shift signal with a quartz crystal electrode [189]. A resonance frequency is produced on the quartz crystal between two nanostructured (gold) plates to detect the food pathogens [190]. A SAW biosensor is a flexible biosensor that is reliable, reproducible, and wirelessly controllable. This sensor produces acoustic waves on the piezoelectric surface and measures the changing acoustic wave to detect pathogens [191].

3.5.3.2. Magnetostrictive biosensor. A magnetostrictive biosensor is a recent technique to detect pathogens in food samples. It consists of a magnetoelastic resonator with oligonucleotide probes that bind to the specific pathogen. The action of magnetostriction of mechanical resonance produces a resonant frequency, and signals are detected and recorded by the analyzer [186]. The advantage of this sensor is that it is rapid, cost-effective, and efficient for mass production to detect foodborne pathogens [192].

3.6. Advanced biosensors for the detection of foodborne pathogen

Above, biosensors are used for the fast and specific detection of foodborne pathogens from food samples. There are some limitations in high cost, stability, reproducibility, and instrumentation. Advances in nanomaterial technology and interdisciplinary multifunctional development of these biosensors to detect pathogens in the food [140]. Nanomaterial-based biosensors, microfluidics-based biosensors, aptamer-based biosensors, portable instrumental biosensors, and smartphone-based biosensors are the rapid and sensitive advanced biosensors for on-site and end-user-accessible devices to detect pathogens.

3.6.1. Nanomaterial-based biosensor

A nanomaterial-based biosensor is a novel biosensor with electricity, optics, mechanics, and thermal properties developed as a sensing platform for detecting pathogens in food samples [193]. It provides rapid, portable, sensitive, and direct on-site detection of the target pathogen [194]. The nanomaterials that are used in this sensor are (1) metallic nanomaterials-gold (AuNPs), silver (AgNPs), platinum (PtNPs), and palladium (PdNPs); (2) metal oxide nanomaterials: erium dioxide (CeO_2) and copper oxide (CuO); (3) magnetic nanomaterials (MNPs): as NiO, CO_3O_4, and Fe_2O_3; (4) carbon nanomaterials-carbon nanotubes (CNTs) and graphene; (5) polymer nanomaterials: dendrimers, conducting polymers, and molecularly imprinted polymers; (6) quantum dots (QDs), (7) upconverting nanomaterials (UCNPs), (8) transition metal dichalcogenides (TMDs), and (9) carbon nanomaterials [195]. This class of nanomaterials can interact with target pathogens to produce a measurable signal [196]. *Listeria monocytogenes, Salmonella enterica,* and *Vibrio parahaemolyticus* are detected by a metal-organic framework (MOF-NSs) nanosheet where target DNA is labeled with fluorescence. The detection limits are 35 pM, 28 pM, and 15 pM for the gene segments of *Listeria monocytogenes*, *Salmonella enterica,* and *Vibrio parahaemolyticus,* respectively [197].

3.6.2. Microfluidics-based biosensor

A microfluidics-based biosensor is an advanced biosensor that is reproducible, controllable, and scalable for detecting foodborne pathogens [198]. It is also called a micro total analysis system (mTAS) or lab-on-a-chip (LOC). A microfluidics-based biosensor has a micropump, micromixer, microvalve, and detector components to produce a signal [199]. This biosensor consists of fluid microchannels which control the fluid flow through force and pressure for sampling, mixing, separation, and detection of pathogens in a single chip [200]. *Salmonella* spp. serogroup are detected by microfluidic biosensor devices from poultry products, where three microchannels imply three focus regions for concentrating samples and three sensing regions for detecting pathogens. Samples from the poultry are introduced to the biosensor through an antigen inlet. Pathogens are focused on the center of the microchannel and pressed toward the sensing region by dielectrophoresis force. An impedance analyzer targets *Salmonella* spp. with a detection limit of 7 cells/mL in 40 min [201].

3.6.3. Aptamer-based biosensor

Aptamer-based biosensors employ aptamers (DNA or RNA molecules) as recognition elements [202]. It is based on optical, electrochemical, and mass transduction, where aptamers bind targets such as proteins, carbohydrates, peptides, small molecules, toxins, metal ions, and live cells with high specificity and affinity [203]. Systematic evolution of ligands by exponential enrichment (SELEX) selects the oligonucleotides for aptamers. The SELEX process is done in two steps-firstly, polymerase chain reaction (PCR) amplifies the real oligonucleotides at the desired concentration. Secondly, amplified products are incubated with a specific target. This technique provides aptamers with high affinity and specificity to target molecules [204]. The advantages of the aptamer-based biosensor are that it is rapid, inexpensive, easy to synthesize, highly stable in acidic and basic conditions, highly specific, and has a higher affinity to detect the foodborne pathogen in food samples [205]. Aptamer-based magnetic nanoparticles (MNPs) and upconversion nanoparticles (UCNPs) detect *Escherichia coli* in pork samples. The MNPs-cDNA-UCNPs-aptamer conjugate system detects *E. coli* through fluorescence emission with a detection limit of 10 CFU/mL [206]. *Salmonella typhimurium* is detected by the aptamer biosensor-based dual signal amplification system (surface-enhanced Raman scattering) and hybridization chain reaction (HCR) with a limit of detection of 6

CFU/mL in 3.5 h [207]. An aptamer-based colorimetric technique is used to identify *Staphylococcus aureus* with a detection limit of 81 CFU/mL in a 5.5 h [208].

3.6.4. Portable instrumental biosensor

A portable instrumental biosensor is a device with lightweight, reduced size, and reliable correspondence of interface [209]. This biosensor measures electric signals in the incubation chamber of a pathogen sample where the temperature of the target pathogen and the electric measurement are regulated by a thermo-regulated detector [210]. A portable biosensor develops a rapid, cheap, accurate, and direct detection device to detect foodborne pathogens [211]. A personal glucose meter (PGM) is used to detect *Staphylococcus aureus* in food samples by a portable biosensor with a limit of 2 CFU/mL [212]. A portable biosensor based potentiometric is used to detect *E. coli* O157:H7, which measures the analytical data by applying an ion-sensitive electric field [213].

3.6.5. Smartphone-based biosensor

A smartphone-based biosensor is based on an optical sensor that depends on the micro-imaging sensor of the smartphone camera to image the pathogen [214]. The operating system consists of a high sensing micro quality camera, operational drive systems, internal memory, and communication and GPS modules for portable detection of foodborne pathogens [215]. This sensor develops three sensing aspects-microscopic imaging, colorimetric, and luminescence by smartphone sensing systems. Smartphone-based biosensors are among the most advanced biosensors because of their simplicity, rapid selection, portability, reduced size, and low cost for detecting pathogens [216]. *Escherichia coli* O157:H7 is detected by a smartphone-based colorimetric aptasensor system in milk with a detection limit of 5.24×10^2 CFU/mL in 1 h. A smartphone-based colorimetric device detects the pathogen by analyzing chromogenic results using acrylic plates, a light-emitting diode, and a portable power pack [217]. A smartphone-based fluorescent microscopic biosensor detects *Salmonella* spp. through a microfluidic chip using a microscopic fluorescent APP system with a detection limit of 58 CFU/mL [218]. A smartphone-based portable amperometric immunosensor detects the hepatitis B virus with a limit of 0.17 μg/mL [219]. However, optimizing lighting conditions is challenging in the smartphone-based biosensors [220].

3.7. CRISPR-cas-based molecular diagnostics for the detection of foodborne pathogen

The clustered regularly interspaced short palindromic repeats-associated nuclease (CRISPR-Cas protein) may be able to overcome the limitations on nucleic acid detection mentioned above due to their several benefits, such as the ability to distinguish between single nucleotide polymorphisms (SNPs), recognize targets at physiological temperatures, and perform fast detection with exceptional sensitivity and specificity [221]. Therefore, food safety, environmental contamination, life science, and other areas have all been seen using CRISPR-Cas-based diagnostics [222]. Also, new diagnostic techniques have been employed by combining Cas proteins with other technologies such as protein aptamers, isothermal amplification, lateral flow, biosensors, biomagnetic beads, biochips, and others are gaining popularity for the detection of foodborne pathogens such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Vibrio parahaemolyticus*, and *Listeria monocytogenes* [223].

The Cas enzyme, which originated in bacteria and archaea to cleave invading viruses' nucleic acids as a kind of defense, is the main component of CRISPR-Cas-based systems. Recently, the CRISPR-Cas-based system has also been used for nucleic acid detection, which has generated a lot of interest [224]. It has been used to modify RNA and genomes. The CRISPR-Cas systems are divided into two classes such as class 1 and class 2, and the class 2 system includes Cas9, Cas12, Cas13, and Cas14, which make up the most common toolbox for nucleic acid detection [225]. In most studies, the CRISPR protein is Cas9, which may be paired with other methods to create versatile methods for pathogens detection. Nevertheless, specific labeling of samples is always necessary to signal the readout of the detection [221]. Cas12 can directly target dsDNA without further processing the amplified sequence; however, PAM (Protospacer-adjacent motif) is necessary when targeting nucleic acids [226]. Cas13 is SHERLOCK (Specific high-sensitivity enzymatic reporter unlocking) using RPA (Recombinase polymerase amplification) technology to identify specific nucleic acids within a short time (5 min). Nevertheless, the primary disadvantages of this approach are that the amplification of samples in detection increases the complexity of quantitative analysis and optimizing all reactions in the detection process needs expert biological understanding [227]. Cas14 proteins are efficient for targeted single-stranded DNA (ssDNA) cleavage without involving a PAM for activation. Cas14 demonstrated a lower tolerance for nucleotide base mismatches between the target and crRNA than Cas12, making it an effective tool for the highly precise detection of SNPs [228].

4. Conclusions

This review discusses conventional and advanced detection methods for foodborne pathogens. Early detection of pathogens in food is essential to ensure food safety and avoid harmful food poisoning. Conventional methods are solely laboratory-based. The disadvantage of the conventional method includes being time-consuming, the intense requirement of resources, a risk of contamination, and the skilled workforce required to obtain better results. At the same time, the advantages of advanced methods include faster response, simplicity, strong intensity, low cost, and rapid data analysis. However, both the conventional and advanced techniques have advantages and disadvantages; therefore, during method selection, the adopted method must be accurate, reliable, cost-effective, and selective for a particular foodborne pathogen and fast enough to attain consistent results. Aside from that, some challenges remain in the industrial-scale application, such as rapid, reliable sample preparation processes and smart detection techniques for foodborne pathogens. Furthermore, the innovation of new devices for detecting harmful pathogens depends on the food type and nutritional components (protein, fat, fiber, and carbohydrates) of food. Therefore, specific sample preparation methods and analytical tools are

needed to identify pathogens in each food product. Future research should be based on these analytical drawbacks to develop a suitable technique for comprehensively detecting foodborne pathogens. Additionally, potential attributes such as precision, accuracy, validation, environmental friendliness, cost-effectiveness, and utilization on a commercial scale should be considered to achieve a unique and reliable detection method.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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