Chapter

Tools for Rapid Detection and Control of Foodborne Microbial Pathogens

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Abstract

Foodborne illnesses have become more common over time, posing a major threat to human health around the world. Foodborne pathogens can be present in a variety of foods, and it is critical to detect them in order to ensure a safe food supply and prevent foodborne illnesses. Traditional methods for detecting foodborne pathogens are time-consuming and labor-intensive. As a result, a range of technologies for quick detection of foodborne pathogens have been developed, as it is necessary for many food analysis. Nucleic acid-based, biosensor-based, and immunological-based approaches are the three types of rapid detection methods. The ideas and use of modern quick technologies for the detection of foodborne bacterial infections are the focus of this chapter.

Keywords: foodborne illness, microorganisms, detection, traditional techniques, molecular methods

1. Introduction

Food poisoning, often known as foodborne illness, is caused by consuming infected food or beverages. Foodborne diseases are to blame for global morbidity and mortality. The gastrointestinal tract of the consumer is the primary organ affected by food infections, but few can target the neurological system, brain, or spinal cord. The researchers discovered more than 250 foodborne illnesses. During 2009–2015 Foodborne Disease Outbreak Surveillance System (FDOSS), received reports of 5760 outbreaks that resulted in 100,939 illnesses, 5699 hospitalizations, and 145 deaths in Columbia. Outbreaks caused by Listeria, Salmonella, and Shiga toxin-producing *Escherichia coli* (STEC) were responsible for 82% of all hospitalizations and 82% of deaths reported [1]. Food poisoning is usually caused by several forms of fungi, bacteria, viruses, and parasites. Food poisoning is thought to be caused by harmful toxins from both microbial and non-microbial sources. WHO estimates that in 2010, with a world population of 6.9 billion, global foodborne diseases resulted in 600 million illnesses, 420,000 deaths. Globally, animal-source foods—meats, fish, dairy products, and eggs—account for approximately one-third of the total burden of foodborne disease [2]. The rapid and precise monitoring and detection of foodborne pathogens

are some of the most effective ways to control and prevent human foodborne infections. Traditional microbiological detection and identification methods for foodborne pathogens are well known to be time-consuming and laborious, as they are increasingly being perceived as insufficient to meet the demands of rapid food testing. Thus, there is a need for novel methods that can detect close to "real-time", small numbers of viable bacterial cells within a given volume of food. Recently, various kinds of rapid detection, identification, and monitoring methods have been developed for foodborne pathogens, including nucleic-acid-based methods, immunological methods, biosensor-based methods, etc. The application of biosensor technology offers promising solutions for portable, rapid, and sensitive detection of microorganisms in the food industry. To limit the spread of foodborne pathogens and outbreaks of foodborne illness, rapid, accurate, and reliable methods of identifying foodborne microbial pathogens are required [3].

1.1 Global burden of food borne disease

FBD is expected to cause 76 million illnesses, 325,000 hospitalizations, and 5000 fatalities in the United States per year, as well as 2,366,000 cases, 21,138 hospitalizations, and 718 deaths in England and Wales. Foodborne Disease Outbreak Surveillance System (FDOSS) received reports of 5760 outbreaks in Columbia between 2009 and 2015, resulting in 100,939 illnesses, 5699 hospitalizations, and 145 fatalities. Listeria, Salmonella, and Shiga toxin-producing *Escherichia coli* (STEC) outbreaks were responsible for 82 percent of all reported hospitalizations and deaths. With a global population of 6.9 billion people, WHO estimates that global foodborne infections caused 600 million illnesses and 420,000 deaths in 2010. Animal-source foods, such as meats, fish, dairy products, and eggs, account for almost one-third of the entire burden of foodborne disease worldwide (**Figure 1**) [2–6].

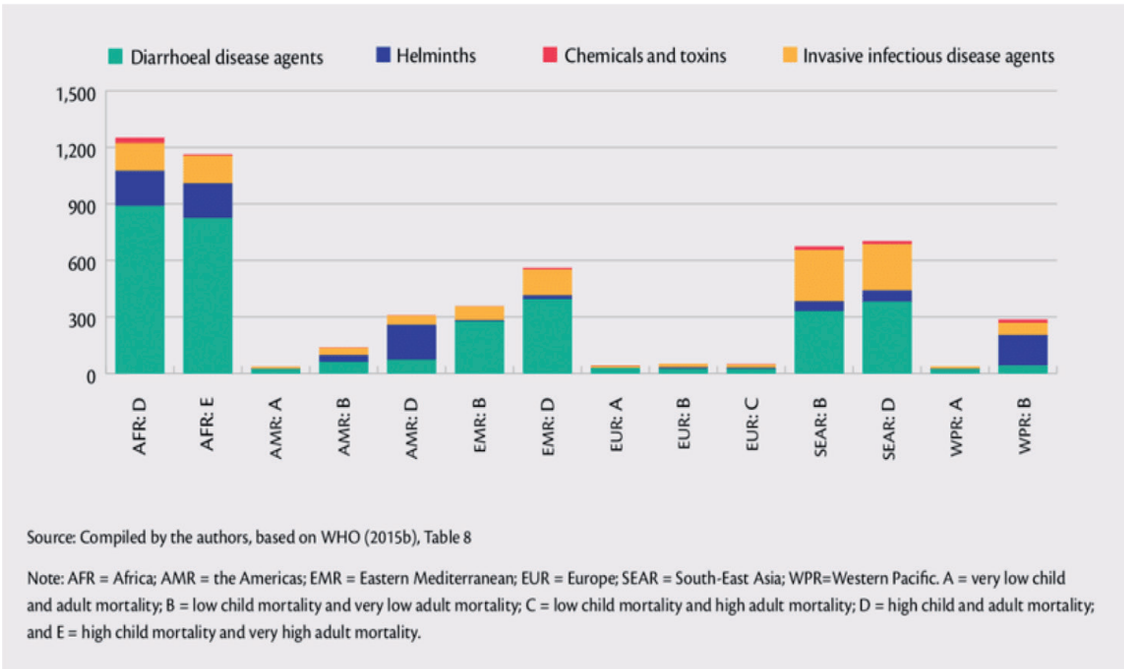

Figure 1.
The global burden of foodborne disease (DALYs per 100,000 population) by hazard groups and by subregion (Food systems and diets: Facing the challenges of the twenty first century, Lukasz Aleksandrowicz, Publisher: Global Panel on Agricultural and Food Systems for Nutrition).

2. Foodborne illness (causes and symptoms)

Food can be contaminated by infectious organisms or their poisons at any step during processing or manufacture. Food contamination can occur at any stage of production, including growing, harvesting, processing, storage, transporting, and preparation. Cross-contamination is a common cause, with hazardous organisms being transferred from one surface to another. Food poisoning can also be caused by eating raw or undercooked meat and poultry, seafood, or raw shellfish [7]. Nausea, vomiting, watery or bloody diarrhea, stomach pain and cramps, and fever are the most prevalent symptoms. The majority of symptoms are gastrointestinal, although they can also manifest as neurological, gynecological, malignant, and immunological disorders.

3. Pathogens causing foodborne illness

Foodborne pathogens are mainly bacteria, viruses, or even parasites that are present in the food and are the cause of major diseases such as food poisoning. **Table 1** showed the various common pathogens causing foodborne illness and its symptoms.

4. Detection methods

One of the most effective strategies to manage and prevent human foodborne diseases is to monitor and detect foodborne pathogens quickly and precisely. Traditional microbiological detection and identification procedures for foodborne pathogens are well known for being time-consuming and labor-intensive, and they are increasingly being seen as unable to fulfill the demands of rapid food testing. As a result, new approaches are needed to detect small quantities of viable bacterial cells in a given volume of food in near real-time. For foodborne pathogens, several types of quick detection, identification, and monitoring technologies, such as nucleic-acid-based methods, immunological methods, and biosensor-based methods, have recently been developed. Occasionally, false-negative or false-positive results are obtained, necessitating further investigation. ELISA is a very reliable and precise method for detecting a wide range of proteins in a complex matrix in both qualitative and quantitative terms. The use of biosensor technology in the food business offers promising solutions for portable, quick, and sensitive detection of microorganisms. The straightforward and easy-to-use immunomagnetic separation of *E. coli* O157:H7 employing aptamers-gold nanoparticle probe quenching Rhodamine B's fluorescence was performed. **Figure 2** showed the various detection methods of foodborne pathogens by conventional and novel strategies.

Food microbiological testing has always been an important aspect of the food production process, but it is most commonly used for end-product control. Microbiological testing has two main goals: determining the absence of pathogens or their toxins to ensure food safety, and determining the overall microbial load to determine product quality and shelf-life stability.

4.1 Traditional methods

Traditional culture methods cultivate, isolate, and enumerate the target microbe while simultaneously preventing the growth of other microorganisms contained in the

S. no.	Foodborne pathogens or their toxins	Predominant symptoms
1.	*Staphylococcus aureus* and its enterotoxins	Nausea, vomiting, retching, diarrhea, abdominal pain, prostration
2.	*Bacillus cereus* (emetic toxin)	Vomiting or diarrhea, depending on whether diarrheic or emetic toxin present; abdominal cramps; nausea
3.	Norovirus	Nausea, vomiting, watery non-bloody diarrhea, dehydration
4.	*Clostridium perfringens*	Abdominal cramps, diarrhea, putrefactive diarrhea (*C. perfringens*), sometimes nausea and vomiting
5.	*Salmonella* spp., *Shigella* spp., *E. coli*	Fever, abdominal cramps, diarrhea, vomiting, headache
6.	*Vibrio cholearae* (O1 and non-O1), *Vibrio parahaemolyticus*	Abdominal cramps, diarrhea, vomiting, fever, malaise, nausea, headache, dehydration
7.	Enterohaemorrhagic *E. coli*, *Campylobacter* spp.	Diarrhea (often bloody), abdominal pain, nausea, vomiting, malaise, fever (uncommon with *E. coli* O157:H7)
8.	Rotavirus, Astrovirus, enteric Adenovirus	Fever, vomiting, watery non-inflammatory diarrhea
9.	*Yersinia enterocolitica*	Fever, diarrhea, abdominal pain
10.	*Entamoeda histolytica*	Abdominal pain, diarrhea, constipation, headache, drowsiness, ulcers, variable—often asymptomatic
11.	*Taenia saginata*, *Taenia solium*	Nervousness, insomnia, hunger pains, anorexia, weight loss, abdominal pain, sometimes gastroenteritis
12.	*Clostridium botulinum* and its neurotoxins	Vertigo, double or blurred vision, loss or light reflex, difficulty in swallowing, dry mouth, weakness, respiratory paralysis
13.	*Trichinella spiralis*	Gastroenteritis, fever, edema around eyes, perspiration, muscular pain, chills, prostration, labored breathing
14.	*Salmonella tympi*	Malaise, headache, fever, fever, cough, nausea, vomiting, constipation, abdominal pain, chills, rose spots, bloody stools
15.	*Toxoplasma gondii*	Fever, headache, myalgia, rash
16.	*Listeria monocytogenes*, *Campylobacter jejuni*	Fever, chills, headache, arthralgia, prostration, malaise, swollen lymph nodes, and other specific symptoms of disease in question

Table 1.
Various microbial pathogens causing foodborne illness.

food using selective liquid or solid culture media. Pre-enrichment growth, selective enrichment culture, and selective plating are used to identify foodborne pathogens, followed by biochemical identification and serological confirmation of the results. Culture approaches are available in both qualitative and quantitative formats [8].

4.1.1 Culture-based methods

4.1.1.1 Qualitative

When only the presence or absence of a pathogen in a food sample must be determined, qualitative procedures are used, in which presumptive colonies are grown

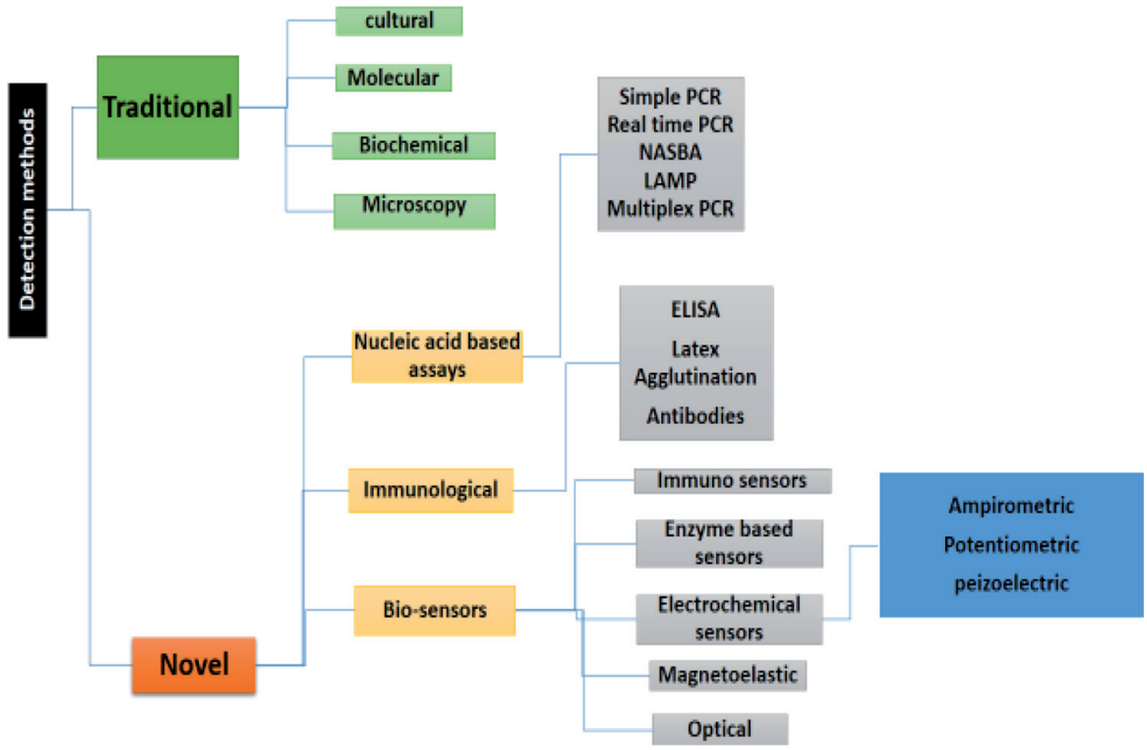

Figure 2.
Various detection methods of foodborne pathogens.

on selective media from a known amount of food. Pure cultures are raised, and the pathogen is identified using various biochemical or serological tests.

4.1.1.2 Quantitative

The plate count method or the most probable number method, both of which are based on serial dilution procedures, are used to count the microorganisms present in the food sample by culture method. Although these methods are reasonably affordable, sensitive, and still considered gold standards, their main disadvantage is their lengthy analysis time and labor-intensive nature. The entire operation usually takes between 7 and 10 days [9–11].

4.1.2 Microscope-based methods

Various approaches based on microscopic and optical traits of the suitably stained microbial cells have been developed for ensuring microbiological safety of foods and food products, these include:

4.1.2.1 Direct epifluorescent filter technique (DEFT)

It is a rapid way of enumerating microbial foodborne pathogens and is used commonly in the dairy sector for raw foods, milk and milk products, beverages, and snacks, among other things. Bacterial cells are captured on the surface of polycarbonate membrane filters, then stained with a fluorochrome like acridine orange and visualized using epifluorescence microscopy. The DEFT count is quick and precise, but it is very labor-intensive and is only useful when there are roughly 10^3 to 10^4 CFU/g of bacteria in the sample [12, 13].

4.1.2.2 Flow cytometry

It is applied to count the number of live bacteria in a sample and to analyze the viability, metabolic status, and antigenic markers of bacteria using fluorescent dyes. When cells are made to pass through a beam of light individually, this technique quantifies their optical properties. The procedure is quick, automatic, and specific if selectively discriminating dyes for specific species of bacteria are available. However, the detection limit for food samples is roughly10^5 to 10^7 CFU/g [13].

4.1.2.3 Solid phase cytometry (SPC)

The principles of epifluorescence microscopy and flow cytometry are combined in this method. Microorganisms are trapped on a membrane filter, fluorescently labeled, and counted automatically using a laser scanner. An epifluorescence microscope coupled to a scanning instrument by a computer-driven moving stage can visually inspect each fluorescent spot. SPC is appropriate only if the count of bacteria is around 10^3 to 10^4 CFU/g [13, 14].

4.1.3 Immunological methods

Antigen-antibody reactions underpin all immunological approaches for detecting foodborne infections. These responses are diverse and specific, but the immunoassay's success is determined by the antibody's specificity. With the emergence of hybridoma technology, monoclonal antibodies that specifically react just to one pathogen have been created. Immunoassays have a detection limit of approximately 10^4 to 10^5 CFU/g [13]. Immunoassays are offered in a variety of configurations.

4.1.3.1 Latex agglutination

Antibody-coated colored latex beads that agglutinate the target antigens (specific pathogen) and generate a visible precipitate are used to identify the food-borne pathogens. The latex agglutination assay is easy and quick, but it is not very sensitive and requires about 10^7 bacterial cells for the reaction to happen [15, 16].

4.1.3.2 Reverse passive latex agglutination test

Antibody-coated colored beads are used to agglutinate soluble antigens or toxins in place of microbiological cells to identify pathotoxins. The inert or carrier particle is attached to a known antibody rather than an antigen in this case. The active site of the antibody must be oriented to face outward. It's used to detect antigens from bacteria like Group A and B Streptococcus, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Neisseria meningitides*, *Haemophilus influenza*, *Cryptococcus neoformans* [17, 18].

4.1.3.3 Immunodiffusion test

Antigen diffusion is allowed through an antibody-impregnated gel, and the formation of a precipitation line shows the presence of a certain foodborne pathogen or antigen.

4.1.3.4 Enzyme-linked immunosorbent assay (ELISA)

It is one of the most widely used and quick methods for detecting foodborne pathogenic bacteria. Most commercially available immunological kits use double antibody sandwich assays, which use commercially available antibody-coated microtiter plates (primary antibody) or other solid matrices to capture antigen (pathotoxin or pathogen) from target food samples, and then add a second antibody (secondary antibody) conjugated with an enzyme to form an antibody-antigen-conjugate "sandwich" [19].

4.2 Nucleic acid-based methods

These methods rely on the detection of certain gene sequences (signature sequences) in the target organism's genotype. The sequences can be chosen to detect a certain group, genus, species, or even strain of the microbe. There are many other types of DNA-based assays, but probes and nucleic acid amplification techniques are the most common and have been commercially developed for identifying foodborne infections.

4.2.1 Nucleic acid probes

Because probe-based tests are simple to apply, they are frequently employed in the food business. Nucleic acid probes are immobilized to inorganic substrates in these experiments so that they can be easily manipulated (e.g., washing out unhybridized DNA) without being damaged or lost. The use of DNA probes is based on a basic idea. It entails the use of a known DNA probe (labeled DNA) to hybridize the DNA sequence of an unknown microbial pathogen. Any microbial cells in the food sample that must be tested for pathogen presence are lysed, releasing their DNA, which is denatured, and the probe is added. The single-stranded DNA probe then hybridizes (annealing of complementary strands) with single-stranded DNA released from pathogenic bacteria present in the food. The signal is obtained by the hybridization of the labeled probe if the desired targeted sequence is present. The probe will not bind if the intended sequence is missing, and no signal will be obtained [15, 16].

4.2.2 Polymerase chain reaction (PCR)

PCR is currently a widely used and incredibly potent technology that allows for rapid exponential amplification of a specific target sequence, reducing the need for culture enrichment. With respect to a single pathogen in food, this approach can detect a single copy of a target DNA sequence. For the identification of microbial infections, PCR provides various benefits over culture and other traditional procedures, including sensitivity, specificity, accuracy, speed, and the ability to detect minute amounts of target nucleic acid in a sample. For the detection of food pathogens, PCR comes in a variety of formats [20, 21].

4.2.2.1 Ligase chain reaction PCR

LCR is a new technology that uses DNA amplification to detect the nucleic acid sequence of bacteria. It's comparable to PCR, except that only probe molecules

amplify by nucleotide polymerization. To make a single probe, two probes for each DNA strand are ligated together. The reaction is driven by thermostable DNA polymerase and a DNA ligase enzyme in LCR. This method has one drawback in terms of food pathogen detection: it can detect DNA from dead species. It is one of the newest amplification approaches for finding point mutations in microbial pathogens. This method uses a thermostable ligase to distinguish between DNA sequences that differ only by a single base pair. LCR assay is commonly used for the specific recognition of *Listeria monocytogenes* [22].

4.2.2.2 Nucleic acid sequence-based amplification (NASBA)

NASBA was created primarily to detect RNA. It's a transcription-dependent amplification technique that uses promoter primers to recognize specific target sequences and synthesize RNA amplicons. Reverse transcriptase, Rnase H, and T7 RNA polymerase are three viral enzymes that work together to amplify RNA targets. A primer attaches to the target RNA sequence, and a reverse transcriptase produces a cDNA strand. The template RNA is subsequently digested by RNase H, and a second primer binds to cDNA, allowing the reverse transcriptase to generate double-stranded cDNA. Finally, T7 RNA polymerase is used in an amplification process to generate RNA transcripts. The main advantage of this approach is that it is isothermal, which eliminates the need for expensive thermal cyclers., NASBA diagnostics were originally developed for the identification of viruses, but they have also been used in food testing to detect *E. coli*, Campylobacter, *Salmonella enterica*, and *L. monocytogenes*, in a variety of foods, as well as *Cryptosporidium parvum* in water [23, 24].

4.2.2.3 Strand displacement amplification

Strand Displacement Amplification (SDA), is an isothermic amplification technique that uses four different primers, each with a Hind II exonuclease restriction site, DNA as a template, and an exonuclease-deficient fragment of *E. coli* DNA polymerase 1 (exo-Klenow) for primer elongation. About 10^9 copies of target DNA can be made in a single reaction. SDA is the basis for commercial detection assays like BD Probe Tec, and it has recently been tested for detecting *Mycobacterium tuberculosis* directly from clinical samples [25, 26].

4.2.2.4 Nested polymerase chain reaction

Nested polymerase chain reaction is a revision of polymerase chain reaction. Because it decreases non-specific binding in products caused by the amplification of unanticipated primer binding sites, this approach is more sensitive and specific than traditional PCR. Two sets of primers are utilized in two separate polymerase chain reaction runs in this procedure. The first set of primers is used to amplify a target sequence, which is subsequently employed as a template for a second amplification. The second primer set remains internal to the first amplicon. As a result, if the first amplification is nonspecific, secondary amplification does not occur. This innovative PCR approach has been used to detect a variety of foodborne pathogens, including *Vibrio parahaemolyticus*, *E. coli* O157:H7, Salmonella, *Listeria monocytogenes*, and *Staphylococcus aureus* [19]. NPCR was used to identify contamination of *Fusarium culmorum* in cereal samples [27].

4.2.2.5 Real-time PCR

Real-time polymerase chain reaction, also known as a quantitative real-time polymerase chain reaction, is a technique for amplifying and quantifying a specific DNA molecule in real-time. It has the ability to detect as well as quantify. The quantity can be either a perfect number of copies or a relative amount when normalized to DNA input or additional normalizing genes. The amplified DNA is detected in real-time as the process progresses. In real-time PCR, there are two main methods for detecting products: (1) non-specific fluorescent dyes, such as SYBR-green I, EtBr, and others, that intercalate with any double-stranded DNA. (2) Sequence-specific DNA probes, such as TaqMan, Molecular Beacons, Scorpions, and others, are comprised of oligonucleotides tagged with a fluorescent reporter that allows detection only after hybridization of the probe with its complementary DNA target. For the identification and characterization of food-borne diseases, a number of commercial kits based on real-time PCR technology are now available on the market [28–30].

4.2.2.6 Multiplex polymerase chain reaction

In the food industry, the expense and restricted volume of test samples are the most important factors to consider when evaluating quality. Multiple sets of primers are included in a single reaction tube in multiplex PCR, allowing more than one target sequence to be amplified in a single reaction system. A single test run can yield more information if numerous genes are targeted at the same time. The main benefit is that less reagent and enzyme (Taq DNA polymerase) are used. Another advantage is that, because pathogens are evaluated individually, sample preparation and findings are completed in a short amount of time. The only drawback is that amplified fragments of the same length cannot be distinguished, and a smaller amount of amplified product may not show up on an agarose gel. This could be solved by developing primers that are longer and have a higher melting temperature (Tm) than those used in conventional PCR [22, 24].

4.2.2.7 Low-stringency single-specific-primer PCR

Low-stringency Single-Specific-Primer PCR (LSSP-PCR) is a straightforward PCR technique for detecting single or multiple mutations in gene-sized DNA fragments. The first of the two steps are specific PCR (sPCR), which is used to obtain the DNA template, and the second is LSSP-PCR, which uses low-stringency conditions, and only one primer, which is commonly utilized in the sPCR. It's utilized to identify infectious pathogens like the Human Papilloma Virus (HPV), Trypanosoma cruzi, Trypanosoma rangeli, and Leishmania infantum by their genetic typing [30, 31].

4.2.2.8 Restriction fragment length polymorphism

Restriction Fragment Length Polymorphism (RFLP) is a very simple approach that involves the digestion of genomic DNA with certain restriction enzymes. It is used to compare the number and size (mass) of fragments produced by restriction endonucleases cutting at a certain recognition site of the target DNA molecule. Electrophoretic separation is used to examine the resultant DNA fragments. Presence, absence, or changes in the weight of the resultant DNA fragments are evidence of altering DNA sequences. For the species-level differentiation of bacteria,

this approach requires pure culture. For the accurate detection of Staphylococcus and Listeria spp., RFLP in conjunction with PCR has been employed [32, 33].

4.2.2.9 Amplified fragment length polymorphism (AFLP)

Another genotyping approach is AFLP, which is based on the selective amplification of restriction segments of DNA molecules. The approach comprises digestion of whole pure genomic DNA using restriction endonucleases, followed by ligation of the resultant fragments with a double-stranded oligonucleotide adaptor complementary to the restriction site's base sequence. Primers corresponding to the contiguous base sequences in the adaptor, the restriction site, and one or more nucleotides in the original target DNA are used to selectively amplify sets of these fragments in PCR. Gel electrophoresis is used to examine the PCR-amplified DNA fragments. AFLP can be used to identify contamination sources, especially in cases involving live stocks [34, 35].

4.2.2.10 Random amplified polymorphic DNA technique

The Random Amplified Polymorphic DNA (RAPD) approach is a PCR experiment that can be used to distinguish races, strains, and pathogenic or non-pathogenic isolates using arbitrary primers. The primers used in this procedure are very short bits (10 or fewer bases) of DNA from a known source. It's very likely that these primers will be able to discover some complementary sequences in the target DNA, resulting in a variety of different-sized DNA fragments. When the results of such a reaction are studied using gel electrophoresis, various banding patterns emerge, some of which may be unique to certain species, varieties, or strains. Some pathogenic fungi may be detected and diagnosed using the patterns alone [36].

4.2.2.11 Loop-mediated isothermal amplification (LAMP)

The loop-mediated isothermal amplification (LAMP) approach for detecting target genes in food samples is a fast, accurate, and cost-effective method. Under isothermal circumstances, LAMP is a single-step amplification reaction that amplifies a target DNA sequence with high sensitivity and specificity. LAMP approach involves three phases, an initial phase, a cycling amplification step, and an elongation step. It uses a strand-displacement DNA polymerase, as well as two inner primers and two outer primers that recognize six different locations inside a target DNA. Because the amplification reaction happens only when all six areas inside a target DNA are appropriately recognized by the primers, the LAMP test is exceedingly specific. Visual judgment, rather than post-amplification electrophoresis, simplifies detection. LAMP has been used to detect a variety of pathogens that cause foodborne illnesses. LAMP kits have been commercially produced for detecting Legionella, Salmonella, Campylobacter Listeria, and verotoxin-producing *Escherichia coli* [37, 38].

4.2.2.12 Repetitive extragenic palindromic PCR

Because of the many typing methods employed in terms of time, accuracy, and cost, rep-PCR may be able to obtain rapid, accurate, and higher resolution findings among the various strains involved in the hospital outbreak. REP PCR was recently

utilized in Italy to identify and classify *Ochrobactrum anthropi* strains. During their hospitalization at Catanzaro University Hospital (Italy) Oncology O.U Institution, all of the patients became infected. There had never been any cases of *O. anthropi* infection before. This was a more precise, efficient, and strong tool for bacterial typing and monitoring, as well as nosocomial infection control [39, 40].

4.2.2.13 DNase treated DNA (DTD) PCR

The key benefit of this technology is that it avoids the issues that PCR-based techniques have with the quick detection of foodborne pathogens. The challenge of testing a single organism at a time was solved by using a multiplex PCR approach. To discover a solution to the problem of false-positive results acquired by amplification of DNA from dead cells, DNase I enzyme treatment followed by PCR (that is DTD-PCR) was tried. When the multiplex procedure was examined for specificity, no interferences or non-specific amplification were identified. As a result, this DTD multiplex PCR technique may be practically used to identify viable cells of four major pathogens, including *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella enterica*, and *Vibrio parahaemolyticus* [41].

4.3 Nanoparticles in pathogen detection

The manipulation of matter on an atomic, molecular, and supramolecular scale is known as nanotechnology ("nanotech"). Bacteria, poisons, proteins, and nucleic acids can now be bound to these nanoparticles thanks to advancements in nanomaterial manipulation. One of the most significant advantages of employing nanomaterials for bio-sensing is their enormous surface area, which allows a greater number of biomolecules to be immobilized, increasing the number of reaction sites accessible for interaction with a target species. This characteristic, in combination with strong electrical and optical properties, makes nanomaterials ideal for "label-free" detection and the development of biosensors with higher sensitivity and faster response times. AuNPs (gold nanoparticles) have been employed in a variety of optical and electrical tests. Gold nanoparticles (AuNPs) have been employed in a variety of optical and electrical assays because of their high conductivity. *Salmonella typhimurium* and *E. coli* O157:H7 organisms are detected with AuNPs at concentrations of 98.9 cfu/mL and 1–10 cfu/mL, respectively. Quantum dots (2–10 nm) made up of semiconducting fluorescent nanoparticles with a semiconductor material core (often cadmium combined with selenium or tellurium) and an additional semiconductor shell (typically zinc sulfide) detected *E. coli* 0157:H7 10^3 cfu/ml (brain, heart infusion broth). Multi-walled nanotubes (MWNTs, 2–100 nm) are simply a number of concentric single-walled nanotubes (SWNTs, 0.4–3 nm) that exhibit photoluminescence and have good electrical properties; semiconductors are employed for the detection of *E. coli* O157:H7 at the 1 cell/mL limit. The detection of dsDNA and ssDNA was attempted using non-functional AuNPs. Citrate-coated AuNPs in this approach has a distinctive red color in the colloidal state. The addition of salts can easily cause AuNPs to aggregate, resulting in a purple color; the change in color can be seen with the naked eye. The negatively charged AuNPs interact electrostatically with ssDNA, which can uncoil in such a way that its hydrophilic negatively charged phosphate backbone is exposed to aqueous solutions, and DNA bases interact with the AuNPs surface via VanderWaals forces, giving the AuNPs a negative charge and increasing their repulsion. These characteristics have been used to create a biosensor

that can detect a PCR product in the same tube within minutes. In comparison to existing approaches, the created biosensor is very selective and sensitive, and it can detect low levels of DNA [42–44].

4.4 Biosensors

Pathogenic microbes have been found in common foods such as milk, cheese, pork, chicken, raw vegetables, and fruits. The pathogens can be determined using standard procedures in about 1–2 days. Biosensors are the most promising new tool for combating this problem. A biosensor is an analytical instrument that translates biological signals and responses into electrical signals. This comprises two key components: a bioreceptor that recognizes the event and a transducer that converts the recognition event into a quantifiable sensitive electrical signal. A bioreceptor can be a microbe, organelle, cell, tissue, antibody, enzyme, nucleic acid, biomimic, or a combination of the above, and the transduction can be thermometric, electrochemical, optical, piezoelectric, or magnetic.

4.4.1 Immunosensors

Biosensors based on the interactions of specific antibodies with a specific antigen are known as immunosensors. Antigens detect antibody binding by immobilizing the reaction on the surface of a transducer, which translates surface change parameters into detectable electrical impulses. Bioreceptors can be monoclonal, polyclonal, or recombinant, depending on their qualities and the method they are synthesized. In eggs and chicken meat, a sandwich immunoassay was developed for two Salmonella species (*S. gallinarum* and *S. pullorum*). According to the researchers, a linear response to Salmonella species was found in the concentration range of 10^4 to 10^9 CFU/ mL, and the detection limit for both species was 3.0 10^3 CFU/mL. Xu et al. investigated immunosensors functioning with screen-printed interdigitated microelectrode (SP-IDME) transducers [39]. The immunosensor was capable of identifying E. coli O157:H7 and *S. typhimurium* in pure culture samples at concentrations of 10^2 to 10^6 CFU/mL, according to their findings. For the detection of *S. typhimurium* in milk, a cadmium selective polymeric membrane microelectrode (Cd-ISE) was used as a transducer. The detection limit was discovered to be 2 cells per 100 μL. In their study, they found that the average total time per assay for detecting *S. typhimurium* in milk samples was 75 minutes [45–47].

4.4.2 Enzyme-based biosensors

On fluorescent and radiolabeled compounds, enzyme as a bio receptor provides a number of advantages. The enzyme immunoassay reagents are non-hazardous, stable, and sensitive. By immobilization, the enzyme bio receptor is properly linked to the transducer. Enzymes are chosen based on their unique binding capabilities and catalytic activity, as well as a suitable substrate that allows for enough electron transport to the working. Storage stability, sensitivity, high selectivity, short reaction time, and great reproducibility are all advantages of enzyme immobilization. By labeling the antibody with enzymes, pathogenic bacteria such as *L. monocytogenes*, *E. coli*, and *C. jejuni* can be detected. Horseradish peroxidase (HRP) and beta-galactosidase are the most often used enzymes [48].

4.4.3 Electrochemical biosensors

Biosensors that measure electrochemical responses are known as electrochemical biosensors. They convert the incoming electrical signal directly into an electronic field, allowing small system designs with simple instrumentation to be created. Electrochemical biosensors are categorized as impedimetric, potentiometric, amperometric, and conductometric biosensors. For the detection of *S. aureus*, a simple label-free electrochemiluminescence (ECL) biosensor was created. In that investigation, the ECL intensity declined linearly with *S. aureus* concentrations ranging from 1.0×10^3 to 1.0×10^9 CFU/mL, with a detection limit of 3.1×10^2 CFU/mL. According to the author when a ready-to-use biosensor was used, the entire experiment could be completed in 70 minutes [49].

4.4.4 Amperometric biosensors

Electrochemical processes are studied using amperometric biosensors, which measure current changes in a constant potential. The biosensors' reaction is proportional to the concentration of analyte in a solution. Amperometric biosensors can be set up using two or three electrodes. The researchers developed an amperometric immunosensor for detecting *S. aureus* in food samples. The increase in amperometric response was used to measure the alterations. For *S. typhimurium* detection in milk, an amperometric biosensor was used, which demonstrated qualitative behavior with a very low limit of detection of 101 CFU/mL and a detection time of 125 minutes.

4.4.5 Potentiometric biosensors

Potentiometric biosensors rely on the detection of oxidation and reduction potential of an electrochemical reaction. As a result, a pH meter comprises an immobilized enzyme membrane around the probe, where the catalyzed process produces or absorbs hydrogen ions. Ion-selective electrodes are used in potentiometric biosensors to turn the biological reaction into an electrical signal. Potentiometric biosensors detect potential changes below zero degrees Celsius. Field-effect transistor (FET) devices are used in recent potentiometric devices. Potentiometric biosensors based on carbon nanotubes and aptamers were used to detect *E. coli*, *S. aureus*, and *S. epidermidis* in pigskin, with a working range of 2.4×10^3 to 2.0×10^4 CFU/mL [50]. Piezoelectric biosensors, which work on the idea of directly detecting bacteria without the need for labeling, are fascinating sensors. In general, the bacteria-containing solution is deposited on the surface of the piezoelectric sensor, which is coated with a selective binding agent (e.g. antibodies). As the crystal mass increases, bacteria bind to antibodies, lowering the oscillation frequency. The pathogen (*S. aureus*) was detected in culture and milk using a piezoelectric biosensor, and the results ranged between 4.1×10^1 and 4.1×10^5 CFU/mL [51].

4.4.6 Magnetoelastic biosensors

Magnetoelastic sensors are constructed with amorphous ferromagnetic alloys. Remote sensing is a feature of magnetoelastic sensors, as the signal transmission takes place at a distance from the coil. The materials display a magnetoelastic resonance when triggered by a changing magnetic field, which may be detected using a noncontact signal collection coil. When a target comes into contact with the pathogen alloy

sensor surface, the extra mass creates a shift in the resonance frequency, which the signal collector coil can detect from a distance. Magnetoelastic sensors, as a result, are wireless devices that can be highly useful for remote monitoring. The first wireless biosensors in biosensor platforms are magnetoelastic biosensors. A magnetoelastic biosensor with a working range of 5×10^1 to 5×10^8 CFU/mL was used to detect *S. typhimurium* on the tomato surface [52].

4.5 Pulse field gel electrophoresis

PFGE (Pulse Field Gel Electrophoresis) employs restriction enzymes, which are molecular scissors that cut bacterial DNA at probable restriction sites. These molecular scissors create a DNA imprint that is segregated by size. The bacteria are first placed in an agarose slurry, which is comparable to gelatin, and then the bacterial cell is opened to allow the DNA to be released. Listeria, Salmonella, *E. coli*, and other food-borne pathogens have all been identified using PFGE [45].

4.5.1 Multi locus enzyme electrophoresis (MLEE)

MLST and Multi Locus Enzyme Electrophoresis (MLEE) are useful for determining population structures of non-bacterial haploid infectious agents and for portable molecular typing of those agents that are weakly or strongly cloned. MLEE is commonly used for typing and population genetic analysis of pathogenic fungi and parasites. MLST is a nucleotide-based typing approach that determines a sequence type by analyzing data from housekeeping genes. MLST provides highly selective molecular typing data that can be electronically transferred between laboratories, making it ideal for studying the genetic relationship of bacteria. Some significant pathogens, such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitides*, have been tested with the approach [53, 54].

4.6 Ribotyping

Ribotyping is a DNA-based subtyping procedure in which restriction enzymes are used to break bacterial DNA into fragments. The restriction enzymes utilized include PFGE, which cut DNA into larger pieces, although genomic DNA is cut into a large number of smaller fragments ranging from 1 to 30 kb in size in the ribotyping assay. Electrophoresis is used to separate the pieces according to their size. Furthermore, in southern blotting, DNA probes are selectively bound to target DNA containing genes coding for rRNA synthesis and are hybridized (to probe specific) for the 16S to 23S rRNA genes. For the distinction of *L. monocytogenes* and the characterization of virulence gene polymorphism lineages, automated ribotyping was used [46].

4.7 Plasmid profile analysis

The examination of plasmid DNA profiles has been utilized to type a variety of gram-negative and gram-positive bacteria. In the sphere of health care, it is applied and employed as a marker for comparing strains and assessing the potential spread of a resistance gene. Plasmids are unique in that they can be transferred to another strain by conjugation under selection pressure, but they can also be gained or lost spontaneously during the process. The acquisition or deletion of plasmid causes genetic relatedness to the isolate to become muddled, limiting short-term

epidemiological research. This is particularly beneficial for species such as Staphylococcus spp. and enterobacteria [47].

4.8 Lipidomics

Lipidomics is an emerging active topic of biomedical and molecular research that involves complex lipidome analysis. Lipidome is a quantitative and complete description of a total lipid moiety present in an organism. Thousands of networks in different species, as well as their interactions with other lipids, carbohydrates, proteins, and other moieties, are identified and quantified in vivo. Membrane-lipidomics (description of membrane lipid constituents) and mediator-lipidomics (description of low abundance bioactive lipid constituents) are two subtypes of lipidomics. The study of pathogen lipid profiles is not entirely new. Liquid chromatography and mass spectrometry are driving the study of lipidomics, allowing for the perception, characterization, and quantification of a wide range of lipid classes. Capturing the entire "lipidome" of a cell or tissue in a single experiment is a difficult endeavor. The research will pave the door for the discovery of pathogen-specific metabolic pathways. Cell and molecular biology will be used as a unique way to analyze the intricate lipid signaling during host-pathogen interactions for medication and biomarker development [55].

5. Conclusions

Traditional methods for detecting foodborne pathogens, which rely on culturing methods, are efficient and gold standard methods but time-consuming and labor-intensive. As a result, a variety of quick detection approaches like PCR, application microarray technology, biosensors, etc. have been developed to address the limitations of traditional detection methods. To avoid outbreaks of foodborne diseases and the transmission of foodborne pathogens, quick methods for detecting foodborne pathogens in food products are critical. Traditional methods are less sensitive, specific, time-efficient, labor-saving, and dependable than rapid detection approaches.

Conflict of interest

Nil.

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