

## **Contemporary Approaches to the Detection of Foodborne Pathogens**

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### **Abstract**

Even though food preservation and safety methods have improved a lot, many disease outbreaks caused by foodborne pathogens like bacteria, fungi, and viruses still happen around the world. This shows that these pathogens are still a serious threat to public health. Although there are many reviews on methods for detecting foodborne pathogens, most of them focus mainly on bacteria, even though viruses and other pathogens are equally important. Ready-to-eat (RTE) foods always come in handy and are the surest way in which pathogens in foods are transmitted. Regardless of the hygiene measures in preparation of these foods and the innovations in food preservation techniques and food safety, there is still an increasing number of foodborne outbreaks that have been linked to RTE foods. In recent times, there are lots of research on food-borne outbreaks. Most research focuses on bacteria as the primary source of contaminant with little or no focus on other types of microorganisms like fungi and viruses. Hence, this review will focus on some pathogenic bacteria, fungi and viruses that have been linked to foodborne outbreaks. This review will shed more light on how culture-based methods, application of immunoassay methods and nucleic-acid-based PCR are useful in the detection of foodborne outbreaks. It provides substantial information on how different methods are used in the detection of foodborne diseases.

**Keywords:** Foodborne; Pathogens; Diseases; PCR; Immunoassays; Culture-Based; NGS.

### **1.0 Introduction**

Microorganisms are used to make some foods and food products, but they can also cause food to spoil. During food production, if harmful microorganisms come into contact with food, the food can become unsafe to eat. Microbial interaction with food could be from different sources; it could be from nature (environment), hygiene of the food handlers or the preservation method. All these factors affect food safety and can cause food spoilage (Lulietto *et al.*, 2015; Saucier, 2016; Quthama *et al.*, 2022). Regardless of the fact that there has been a great improvement in food preservation methods, which has helped to increase the shelf life of RTE foods and reduce the rate of spoilage, there is still a risk of

contamination and food poisoning if proper hygiene and handling are not followed. It is commonly known that food preservation helps to get rid of food pathogens, but there have been several studies that show that eventually, when these pathogens are exposed to environmental stress, there is a possibility that they will survive as dormant cells and are often regarded as viable but not culturable cells (VBNC) (Foddai & Grant, 2020). Diseases caused as a result of a foodborne outbreak pose a huge risk to human lives. Worldwide, there have been over 250 foodborne diseases that have been recorded (Mead *et al.*, 1999) with *Salmonella* spp., *Clostridium botulinum*, *Campylobacter* spp., *Staphylococcus aureus*, *Listeria monocytogenes* (Martnovic *et al.*, 2016; Bintis, 2017; Lianou *et al.*, 2023), and *Vibrio* spp. (Adebisi *et al.*, 2023) topping the list. As a result of the recurrent outbreaks, several detection methods have been developed for the detection of these pathogens. Notably, aside from bacteria being the most common foodborne pathogens, foodborne diseases caused as a result of fungal and virus species have also been identified with *Penicillium*, *Aspergillus* and *Fusarium* topping the list of the fungal species (Martnovic *et al.*, 2016) and Rotavirus, Norovirus, Hepatitis A and E virus and Adenovirus topping the list of the virus species (Pexara & Govaris, 2020; Lianou *et al.*, 2023). The consistent recurrence of foodborne diseases explains that, regardless of the advancement in food preservation and safety, foodborne diseases still pose a huge threat to public health (Foddai & Grant, 2020). Thus, different methods have been developed to isolate these pathogens in food and reduce the risks of outbreak including Polymerase Chain Reactions (PCR) {Reverse Transcriptase PCR (RT-PCR), Real-Time PCR, Multiplex PCR} DNA microarray, Nucleic acid sequence-based amplification (NASBA) and Next Generation Sequencing (NGS) immunological and nanotechnology-based methods (Law *et al.*, 2015; Foddai & Grant, 2020; Saravanan *et al.*, 2021).

This review will not only focus on bacteria, which have been tagged the major pathogens of foodborne outbreak but also on fungi and viruses because they are also important foodborne pathogens, as well as prioritising the use of molecular methods like PCR and NGS in the detection of these foodborne pathogens.

## **2.0 Foodborne Diseases Outbreak**

### **2.1 Bacteria**

Worldwide, bacteria have always topped the list of most of the foodborne diseases. Several researchers have reported between 130000 and 400000 people hospitalised and about 6000 deaths in the USA (Mead *et al.*, 1999; Nyachuba, 2010; Ribot & Hise, 2016). In Europe, 21000 people were hospitalised in 2020 as a result of consumption of eggs and egg products contaminated with *Salmonella* (Authority, 2021). *Listeria monocytogenes*, *Campylobacter* and *Yersinia* have also been isolated in Europe (Authority, 2021). In addition, *L. monocytogenes* has been recorded as the major cause of foodborne diseases in 2020 in Europe (Authority, 2021). Notably, it has been recorded that more than 3 million cases of diarrhoea have been linked to foodborne bacteria annually (Ribot & Hise, 2016). The World Health Organization (WHO) has also reported more than 4 million foodborne diseases in Australia (Kirk *et al.*, 2014).

## **2.2 Fungi**

Distinctively, out of about 150 fungal species, only about 300 are foodborne pathogens that pose a huge risk to human lives (Hawksworth, 2001). Although there are several foodborne outbreaks caused by fungal secondary metabolites, such as toxins, in people with extremely low immunity. Nevertheless, people with a suppressed immune system are also prone to foodborne fungal diseases. In the USA, an outbreak of gastroenteritis was reported in 2013 after the consumption of yoghurts contaminated with *Mucor circinelloides*, which left about 250 people hospitalised with severe symptoms of diarrhoea, vomiting and nausea (Lee *et al.*, 2014). Additionally, in Hong Kong, 7 people were diagnosed with food poisoning by *Rhizopus* microspores after consumption of contaminated pre-packaged RTE meals (Cheng *et al.*, 2009). Paterson & Lima (2017) reported that when some filamentous fungi like *Mucor*, *Fusarium* and *Aspergillus* are inhaled or consumed, they can affect the respiratory system and also cause lung damage in immunocompromised individuals. Nonetheless, people with suppressed immunity should avoid the consumption of food contaminated with fungi because the gastrointestinal route is also a passage through which foodborne diseases can occur.

## **2.3 Virus**

Majorly, foodborne disease as a result of virus are not common, but there are some occurrences as well. Viruses like Norovirus and Hepatitis A are the most common foodborne viruses that have been reported in humans. In the USA, Noroviruses have been implicated in about 22 million cases of severe gastroenteritis annually (Miranda & Schaffner, 2019). In Europe, Norovirus was isolated in crustaceans, shellfish, molluscs and has topped the lists of foodborne pathogens in 2020 (Authority, 2021). The largest outbreak was recorded in Europe in 2020, West Nile virus-based diseases topped the list of the foodborne pathogens that left a lot of people hospitalised (Authority, 2021). These enteric viruses (Norovirus, Hepatitis) have been linked to about 14% and 50% cases of foodborne outbreak diseases in Europe and the USA consecutively (Yeargin & Gibson, 2019). In South Korea, during the winter Olympics in 2018, about 195 gastroenteritis cases were reported after the consumption of frozen raspberries (Miranda & Schaffner, 2019). Also, in 2011, the USA reported a viral foodborne outbreak caused by the consumption of contaminated pomegranate seeds, leaving about 165 people hospitalised. Likewise, in 2012, China reported over 1100 cases of Norovirus caused after the consumption of frozen strawberries (Yeargin & Gibson, 2019).

## **3.0 Methods for Detecting Foodborne Pathogens**

In food safety, the detection of harmful pathogens capable of causing diseases in food is essential. Although it always seems like an impossible task because of some external factors like low numbers of target groups, interference by non-target microbiota and difficulties in microbial extraction from food matrices. Several detection methods have been developed in identifying foodborne pathogens, such as culture-based methods, immunological assays, nucleic acid-based methods, polymerase chain reactions and Next Generation Sequencing (NGS) methods. Nonetheless, the accuracy of these methods in detecting the foodborne pathogens lies in the use of suitable aseptic sampling and sample storage protocols. The way samples are collected depends on the type of food, the kind of microbes being

studied, and the method used to detect them. For example, when collecting and testing samples that involve growing microbes, it is important to follow standard rules made by official organizations like the FDA, FSIS/USDA, ISO, and AOAC (Da Silva *et al.*, 2018).

### **3.1 Culture-Based Detection of Foodborne Bacteria**

The culture-based method is often used as the primary method for detecting foodborne pathogens (Chen *et al.*, 2021; Patil-Joshi *et al.*, 2021; Park *et al.*, 2023; Altayb *et al.*, 2023). It involves growing the pathogens on suitable culture media; these pathogens form colonies that can be collected and then subjected to other molecular testing methods. This method often serves as a reference method and is mostly used in food testing laboratories (Foddai & Grant, 2020). The success of this method is dependent on the type of culture media used. It's often advised to use a selective medium as this helps to target the pathogen of choice, thereby eliminating the growth of other pathogens or contaminants (Martinovic *et al.*, 2016). Also, this method makes it possible for the colonies grown on the medium to undergo various testing methods like Gram staining reaction, biochemical reactions, colony characterisation and even the PCR -sequencing method. Although this method is cheap and easy in isolating pathogens, there are several limitations involved. Some pathogens are non- culturable and will not grow on any culture media. Bacteria like *E. coli* can exist as viable but non-culturable when exposed to stress. When this happens, it can lead to a wrong analysis and pose a huge risk to food safety and public health. Furthermore, this method is labor-intensive and requires a step-by-step process, which can be time-consuming and also requires a follow-up process, such as biochemical testing and serological testing. For some bacteria, it takes about a week for detection and it takes a longer time for fungi. This method is not a fast way of detecting pathogens in food (Tiethen & Fung, 1995; Oluwaseun *et al.*, 2018). The majority of the bacteria that have been implicated in the outbreak of foodborne diseases are capable of secreting toxins. Examples of these foodborne bacteria are *L. monocytogenes*, *Clostridium*, *Enterobacteria*, *Salmonella*, *E. coli* and *Bacillus spp.* The culture-based methods are effective in detecting this pathogen except when it's in the VBNC stage. Foodborne diseases as a result of this bacterium occur mostly after the consumption of food contaminated with these bacteria (Lianou *et al.*, 2023). Hence, the culture-based method cannot be completely relied on. But, when the culture-based method is used in addition to other methods like the PCR, Immunoassay, and NGS, the result becomes more reliable (Biswas & Rolain, 2013; Rychert, 2019).

### **3.2 Culture-Based Detection of Foodborne Fungi**

Fungi are capable of producing toxins. These toxins can be inhaled and consumed alongside contaminated food. The culture-based detection of foodborne fungi is time-consuming, as it takes longer to complete. Just like bacteria, the sensitivity of this method becomes reliable when it is paired with other molecular methods like PCR, Immunoassay and NGS. Recently, MALDI TOF MS has become a reliable molecular method of detecting fungi like *Rhizopus*, *Aspergillus*, *Fusarium* and *Mucor* (Elbehiry *et al.*, 2017) because it can quickly identify species-specific proteins that can be matched with reference databases.

### **3.3 Culture-Based Detection of Foodborne Viruses**

The major viruses implicated in the outbreak of foodborne diseases are Norovirus and Hepatitis A. These viruses mostly contaminate seafoods, milk, fish, fruits and vegetables via the faecal-oral route and are transmitted through consumption of contaminated products (Pexara & Govaris, 2020; Su *et al.*, 2021). In cases of Hepatitis E virus, the disease is caused as a result of consumption of raw or semi-cooked meat and liver (Pexara & Govaris, 2020). The use of culture-based methods in detecting foodborne viruses is limited because there is a low risk of viral contamination in food. Also, this method is not effective for fast testing. Then, molecular methods such as the RT-PCR are more accurate for viral detection. In addition, the detection of viruses in food can be demonstrated using the viral quantification method and this is done by using the tissue culture infectious dose 50 (TCID<sub>50</sub>), plaque assay and most probable number method (Bosch *et al.*, 2011).

Notably, Avian Influenza like H7N9 and H5N8 that are considered dangerous to public health have been found in birds, chickens and ducks (Wu *et al.*, 2020; Zhang *et al.*, 2021; Dai *et al.*, 2022). To detect these viruses, samples are mixed and grown in fertilized chicken eggs for a few days. If the sample causes hemagglutination (clumping of red blood cells), it means the virus is present. Sometimes, scientists combine this method with other techniques such as plaque assays or RT-qPCR (Shibata *et al.*, 2018), a test that checks for viral genes. For example, Human adenovirus has been found in foods like lettuce, onions, and strawberries using these combined methods (Marti & Barardi, 2016)

### **4.1 Immunological Assay**

These assays give a precise result in detecting foodborne pathogens and their toxins. It makes use of lateral flow devices (LFD) and enzyme-linked immunosorbent assay (ELISA). These methods are based on the idea that microbial antigens and antibodies attract each other, and this reaction can be used to quickly and accurately detect foodborne pathogens (Oluwaseun *et al.*, 2018). The main benefits of these tests are that they are easy to perform, faster than traditional culture methods, can detect toxins, and are very specific. However, if the test sample becomes contaminated, it can cause false positive results (Priyanka *et al.*, 2016). ELISA and lateral flow devices (LFD) are two of the most common immunoassays used today to detect foodborne microbes and their toxins.

Sometimes, ELISA is combined with other methods such as PCR to make the detection more accurate and efficient (Law *et al.*, 2015; Priyanka *et al.*, 2016; Agriopoulou *et al.*, 2020). For example, the PCR-ELISA method can detect the fungus *Fusarium verticillioides* in corn samples 100 times better than regular PCR (Omori *et al.*, 2018). In bacteria like *Salmonella*, *Campylobacter*, *E. coli* and *Listeria*, ELISA has been used consecutively to detect these bacteria from food. ELISA was used to detect *Listeria* in milk (Tu *et al.*, 2016), *Vibrio parahaemolyticus* in seafood (Kumar *et al.*, 2011), and *Campylobacter* in food samples (Khan *et al.*, 2018). ELISA is often used in detecting aflatoxins and mycotoxins produced by fungi. ELISA has been found effective in detecting this toxin in maize (Hassan *et al.*, 2014), dried stock fish (Ounleye & Olaiya, 2015), peanuts (Oplatowska-Stachwiak *et al.*, 2016) and soymilk (Beley *et al.*, 2013). In addition, Virulence strains of viruses from clinical and environmental samples are detected using ELISA (Kim *et al.*, 2019; Wu *et al.*, 2020).

#### **4.2 Polymerase Chain Reaction (PCR) Methods**

PCR involves the use of a specific DNA or RNA sequence of pathogenic microorganisms. There are different variants of PCR and these variants are nucleic acid-based methods. PCRs are less time-consuming, accurate, consistent and reliable. For each variant, distinct primers are formulated to target the pathogens in food (Priyanka *et al.*, 2016). These primers are added to the nucleic acid from the food samples and it targets the DNA of these pathogens (Singh *et al.*, 2014; Law *et al.*, 2015; Priyanka *et al.*, 2016; Muhamad Rizal *et al.*, 2020). There are three main steps in PCR and it takes about 25-40 cycles. The first step is denaturation, in which the double-stranded DNA is separated into two single strands. Then annealing, in which the distinct primers attach to the complementary sequence on the DNA template and the final step is extension, in which the DNA polymerase adds nucleotides to build a new DNA strand (Singh *et al.*, 2014). The different variants of PCR are: conventional PCR, multiplex PCR and Reverse Transcriptase PCR (Mancini *et al.*, 2016). The conventional PCR has three variants and they are Nested, touch-down and hot-start PCR, which are usually DNA-based. These PCR uses specific primers to target a specific group of microorganisms in food samples (Lee *et al.*, 2014). In bacteria, these methods have been used to accurately detect the presence of *Salmonella* spp., *Campylobacter* spp., and *Vibrio parahaemolyticus* in “keropok lekor” in Malaysia (Adebisi *et al.*, 2023). The Real-Time PCR is often used more than the multiplex PCR because it's able to detect the abundance of specific foodborne pathogens in food samples. For example, *E. coli*, *Campylobacter*, and *Listeria monocytogenes* have been found and measured in foods like cheese, chicken, beef burgers, turkey, pork, eggs, and fish using Real-Time PCR (Gill, 2017; Bai *et al.*, 2022). Notably, the Reverse Transcriptase PCR (RT-PCR) is rarely used in detecting pathogenic bacteria from food because the mRNA is easily degraded in food samples and there is likely a false negative result after the analysis (Xiao *et al.*, 2012). Also, the process is time and labor-intensive (Xiao *et al.*, 2012).

The use of PCR-based methods in detecting fungi in food samples is limited compared to bacteria. Nevertheless, there are several reports of the use of PCR in detecting fungi in clinical and environmental samples (Sexton *et al.*, 2018; Wagner *et al.*, 2018; Luchi *et al.*, 2020; Vergidis *et al.*, 2020). Also, aflatoxigenic *Aspergillus* isolates from ‘meju’, a native food in Korea, gotten from fermented soybean was detected using the multiplex PCR (Kim *et al.*, 2011). In this case, the primers used were distinctly formulated to eliminate the non-aflatoxin-producing fungi from the aflatoxin ones (Kim *et al.*, 2011). It was also used to consecutively detect *Penicillium* and *Fusarium* in contaminated maize powder (Rahman *et al.*, 2020).

In Virus, the PCR methods are usually accurate in detecting viral pathogens in food. The PCR methods like the multiplex PCR, Real-Time PCR, Digital RT-PCR and Qualitative RT PCR is the most used because they are capable of detecting viral pathogens and allow for the quantification of viral pathogens (Miranda & Schaffner, 2019). In most cases, before using PCR, viruses must first be extracted and concentrated from food samples. However, this process can sometimes be inefficient, leading to a low amount or complete loss of viruses (Miranda & Schaffner, 2019).

The PCR test can also be affected by substances in the sample that block the reaction. Another problem with RT-PCR or real-time quantitative PCR is that they cannot tell the difference between infectious and non-infectious virus particles (Sanchez & Bosch, 2016). To solve this, scientists use special dyes such as propidium monoazide (PMA) or ethidium monoazide (EMA) to treat samples before RT-PCR or RT-qPCR. These dyes prevent non-infectious particles from being detected. This method has been successfully used in laboratories to separate infectious Hepatitis A viruses and Rotaviruses from non-infectious ones (Coudray-Meunier *et al.*, 2013). In addition, RT-PCR has been used consistently in detecting viruses in food, e.g., the occurrence of Hepatitis A and Norovirus was isolated in mussels in Italy and this poses a huge risk to the consumers of mussels (La Bella *et al.*, 2017).

### **4.3 Next-Generation Sequencing (NGS) Methods**

These methods, in addition to bioinformatics, are essential detection methods that have improved food safety. The NGS involves two steps: firstly, find the complete DNA sequence of a single microorganism and secondly, in metagenomics i.e. specific groups of microbes can be studied using biological markers like 16S rRNA, to identify the DNA sequence of many microorganisms in one sample (Jagadeesan *et al.*, 2019). The use of NGS coupled with other methods like RT-PCR as a confirmatory technique in detecting bacteria like *S. sonnei*, *L. monocytogenes*, *C. jejuni*, *S. aureus* and *E. coli* has been reported (Leonard *et al.*, 2015). Furthermore, NGS has been used to test ready-to-eat salads and found harmful bacteria like *Aeromonas hydrophila* and *Rahnella aquatilis* (Mira Miralles *et al.*, 2019). When used to read the whole DNA of a germ, NGS is very useful for tracking foodborne pathogens (Moran-Giad, 2017). In fungi, there are limited reports on the application of NGS in detecting fungi in food samples. Nevertheless, there is adequate information on the detection and identification of the pathogen in clinical and environmental samples (Armstrong *et al.*, 2019; Jiang *et al.*, 2022).

In the virus, there are adequate resources for using NGS for the sequencing of foodborne viruses. This explains that, prior to using NGS for sequencing, a non NGS method like RT-PCR will have been used to detect the pathogen from the food sample and then NGS is used as a confirmatory tool e.g. In Germany, there was an outbreak of Norovirus in 2012 after consumption of frozen strawberries, NGS coupled with some variants of PCR was used in detecting the genotype of the Norovirus (Bartsch *et al.*, 2018). Similarly, NGS has also been used to identify viruses in food. For example, many types of Norovirus and Hepatitis A virus were found in organic lettuce, parsley, and strawberries. This was done using a PCR targeting test coupled with NGS that helps detect virus genes in the samples (Itarte *et al.*, 2021).

### **Concluding Remarks and Future Prospects**

This review looked at four main methods for detecting foodborne pathogens: culture-based, immunoassay, PCR-based, and NGS-based methods. Culture-based methods, when combined with tools like MALDI TOF MS and PCR methods (such as Real-Time PCR for bacteria and RT-PCR for fungi), can quickly and accurately identify many foodborne pathogens. PCR and sequencing methods are used more often than immunoassay and NGS methods for detecting pathogens. However, NGS

methods like metagenomics provide very detailed information about the genes, diversity, and behavior of foodborne pathogens. They are also useful for tracking and identifying pathogens throughout the food chain. These modern methods can help detect and control foodborne outbreaks earlier, improving public safety and health. In the future, it is important to train food safety officers to use these tools effectively and to continue research on new methods—such as using bacteriophages—to better control and eliminate foodborne pathogens.

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