Chapter 4
Detection of Microbial Contaminants in Food and Food Products

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ABSTRACT

The detection of microbial contaminants in food and food products is a cornerstone of public health protection and food safety assurance. As foodborne diseases continue to pose a global burden, with pathogens such as Salmonella, Escherichia coli, Listeria monocytogenes, and norovirus accounting for millions of illnesses annually, the need for robust and reliable detection methodologies has become increasingly urgent. This chapter provides a comprehensive overview of the evolving landscape of microbial detection in food systems. It begins by exploring the sources and pathways of microbial contamination across the "farm-to-fork" continuum, highlighting critical control points and microbial risk factors. Emphasis is placed on sampling strategies, including representative sampling, sample preparation, and enrichment

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protocols, which form the foundation of accurate microbial detection. The chapter then examines diverse detection strategies, including culture-based methods, immunological assays (such as enzyme-linked immunosorbent assay and lateral flow tests), and molecular techniques like polymerase chain reaction (PCR), quantitative PCR, loop-mediated isothermal amplification, and next-generation sequencing. Emerging technologies such as biosensors, Clustered Regularly Interspaced Short Palindromic Repeats-based diagnostics, and metagenomics are also discussed for their potential to enhance sensitivity, specificity, and rapidity in pathogen detection. Each technique is assessed in terms of sensitivity, specificity, operational feasibility, and its integration into food safety risk management frameworks. Special attention is given to validation standards, harmonization efforts, and the challenges of deploying these technologies in low-resource settings. The chapter concludes by identifying emerging trends, such as artificial intelligence-assisted detection and portable diagnostics, which hold promise for revolutionizing microbial monitoring in food systems. By bridging microbiological principles with practical applications and regulatory contexts, offering critical insights for researchers, food safety practitioners, and policymakers.

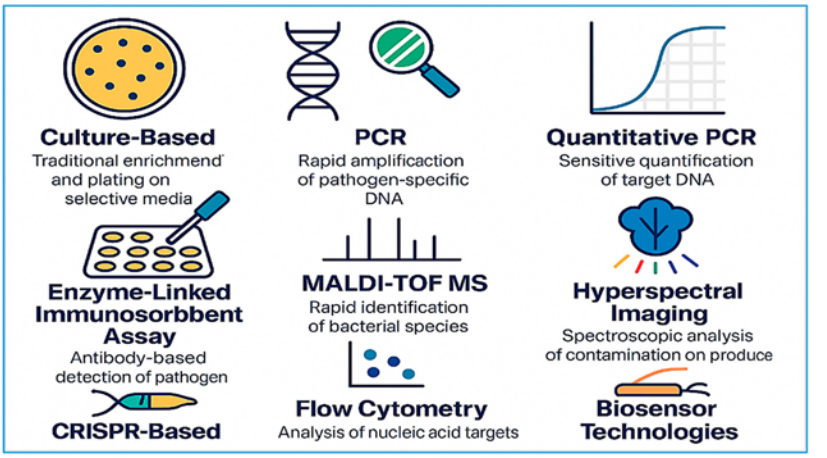
1.0. INTRODUCTION

The detection of microbial contaminants in food is crucial in ensuring public health and food safety. Foodborne pathogens, including *Salmonella, Staphylococcus, Escherichia coli*, norovirus, and *Listeria,* are significant contributors to global health issues, with approximately 600 million illnesses reported annually due to contaminated food (Havelaar et al., 2015; Akkina et al., 2023). The World Health Organization (WHO) highlights that a substantial proportion of foodborne disease burdens can be attributed to animal-source foods, including meats, eggs, and dairy products (Akkina et al., 2023). The continuous rise in foodborne illnesses is often linked to various lapses in food safety practices across the food production and supply chain (Lee and Yoon, 2021).

Detecting microbial pathogens in food may rely on a spectrum of methods ranging from classical microbiological techniques to innovative molecular and sensor-based technologies (Figure 1). Traditional or classical methods, such as culture-based techniques and biochemical tests, remain foundational but are often time-consuming and labor-intensive. Advances in technology have introduced faster, more sensitive approaches, including immunoassays (e.g., Enzyme-Linked Immunosorbent Assay [ELISA]), molecular techniques (e.g., Polymerase Chain Reaction [PCR], Clustered Regularly Interspaced Short Palindromic Repeats [CRISPR]), mass spectrometry (e.g., Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry

[MALDI-TOF MS]), biosensors, and hyperspectral imaging. These modern tools enhance detection accuracy and enable real-time monitoring, thereby supporting more effective prevention and control of foodborne illnesses.

Figure 1. Traditional approaches and cutting-edge technologies for detecting microbial pathogens in food.



Source: *Elbehiry et al. (2025)*

Furthermore, the connection between foodborne illness and effective detection strategies cannot be overstated. Robust detection techniques are essential for identifying the presence of microbial pathogens and preventing outbreaks. Epidemiological data indicate that food contaminated by pathogens results in significant health burdens, with estimates reaching millions of illnesses and thousands of deaths annually in regions such as the United States (WHO, 2015; Bintsis, 2017; US CDC, 2025). The United States Centers for Disease Control and Prevention (CDC) estimates that approximately 48 million episodes of foodborne illness occur yearly in the U.S., emphasizing the importance of effective surveillance and detection strategies to manage food safety (Scallan et al., 2011a; b). The implications of inadequately managed microbial contamination extend beyond individual health risks to encompass broader public health challenges and economic costs. The annual economic burden related to foodborne illnesses in the U.S. alone is estimated to range from $14 to $60 billion (Hu et al., 2022). Thus, there is a critical need to continually advance detection technologies not only to identify contaminants but also to understand emerging patterns in foodborne illness outbreaks (Schomberg et al., 2016).

Modern food safety practices rely heavily on advanced detection methodologies, which incorporate various techniques, including multiplex polymerase chain reaction (PCR), bioinformatics, and whole-genome sequencing. For instance, signifi-

cant advancements in multiplex real-time PCR have enhanced the specificity and sensitivity of pathogen detection, enabling the simultaneous screening of multiple foodborne pathogens from food samples (Kawase et al., 2016). This method has proven valuable in analyzing outbreaks and identifying contamination sources rapidly, as evidenced by its application in monitoring fecal samples during foodborne illness investigations (Oh et al., 2012).

Additionally, rapid detection methods, including genetic and biochemical assays, have facilitated early identification of microbial contaminants, allowing quicker responses to potential outbreaks. The integration of these technologies has transformed pathogen surveillance, enabling the effective tracking and management of epidemics, particularly in environments where the risk of contamination is high, such as meat production and processing facilities (Elbehiry et al., 2022). Elbehiry et al. (2022) emphasize the persistent issues of microbial contamination in meat products and the consequent public health challenges, highlighting the necessity for effective detection strategies tailored to specific food types.

Moreover, the ongoing advancement of detection techniques reflects a growing recognition of the diverse modes of transmission and the complexity of foodborne pathogens. For instance, environmental surveillance and social media-based reporting mechanisms have emerged as innovative strategies to supplement traditional inspection methods. These new approaches enhance detection capabilities by capturing real-time data on food safety incidents, allowing public health officials to respond swiftly to emerging threats (Schomberg et al., 2016). The interplay between food safety practices and detection strategies is evident in the management of foodborne illnesses. The multifaceted nature of food contamination, which can occur at various stages from production to consumption, necessitates comprehensive surveillance systems that integrate detection methods across the entire food supply chain (Havelaar et al., 2015). For example, the integration of consumer reports and health professional feedback into public health databases provides valuable insights into patterns of foodborne illness, directly informing the development of targeted surveillance and detection efforts (Arendt et al., 2013).

Understanding the primary sources of contamination is crucial for designing effective detection strategies. Epidemiological data from the United States Centers for Disease Control and Prevention (CDC) indicate that certain foods, including leafy greens, dairy products, and poultry, are frequently implicated in foodborne illness outbreaks (Scallan et al., 2011a;b). Targeting these food sources with appropriate detection protocols can significantly mitigate the risk of widespread illness. Moreover, technological advancements, such as the use of social media data to monitor foodborne disease trends, have shown promising results in enhancing public engagement and response capabilities (Schomberg et al., 2016). Efforts to improve detection methodologies also play a crucial role in enhancing public awareness of

food safety issues. As consumers become increasingly informed about potential risks associated with their food, effective communication of detection results and food safety information becomes vital (Mayer et al., 2023). There is a higher chance of adherence to recommended food handling and preparation protocols when food safety procedures are more transparent and trustworthy, ultimately reducing the prevalence of foodborne illnesses (Mahunu et al., 2024; Ogwu et al., 2024).

This chapter aims to provide a comprehensive overview of the principles, methods, and emerging technologies used in detecting microbial contaminants in food and food products. It explores traditional and modern detection approaches ranging from culture-based methods to molecular diagnostics, biosensors, and next-generation sequencing, highlighting their applications, advantages, and limitations within various food contexts. The chapter connects fundamental microbiology with practical food safety management by incorporating practical factors such as sampling plans, quality control procedures, and regulatory compliance. It broadens our understanding by elucidating how advancements in digital technologies and rapid diagnostic tools are transforming microbiological surveillance and enabling more proactive responses to contamination events.

2.0. PATHWAYS OF MICROBIAL CONTAMINATION IN THE FOOD CHAIN

Microbial contamination in the food chain is a critical public health concern that impacts food safety and quality at multiple stages, from farm production to consumer consumption. Contamination may occur through a variety of pathways, with specific microorganisms linked to particular sources, each posing distinct risks. These routes are essential for designing effective interventions and mitigating the incidence of foodborne illnesses.

2.1. Sources of Contamination in the Food Chain

The food supply chain is vulnerable to microbial contamination at critical control points: the farm, processing facilities, retail operations, and domestic environments (Table 1). At the farm level, contamination arises predominantly from fecal matter, soil, and water, especially during the irrigation of produce and animal husbandry (Ravaliya et al., 2014; El-Khishin et al., 2017). For example, environmental contamination from fecal sources can introduce pathogens such as *E. coli* and *Salmonella*

to crops during growth or harvesting, directly influencing produce safety (Ravaliya et al., 2014; El-Khishin et al., 2017).

In processing environments, pathogens can be transferred from raw ingredients to food contact surfaces, such as equipment and utensils, thereby facilitating cross-contamination (Zhu et al., 2014; Malavi et al., 2018). Studies have shown that food contact surfaces can harbor harmful bacteria, such as Listeria monocytogenes, which can survive and proliferate on various materials used in food preparation, like stainless steel (Zhu et al., 2014; Zoellner et al., 2018). These surfaces can then contaminate ready-to-eat foods during slicing or packaging processes, leading to significant health risks for consumers.

Retail environments further contribute to microbial contamination through improper food handling practices, inadequate refrigeration, and a lack of sanitization of surfaces and equipment (Krishnasree et al., 2018; Bedane et al., 2022). Improperly cleaned deli slicers, for example, can serve as vehicles for transferring pathogens from contaminated foods to other products, compounding the risk of foodborne illness outbreaks (Koo et al., 2012). Additionally, consumer kitchens often become sites of contamination if safe food handling practices are not followed, highlighting the need for education on food safety (Stavropoulou and Bezirtzoglou, 2019).

Microbial contamination not only poses health risks but also disrupts international food trade and imposes substantial economic burdens due to recalls, import bans, and reduced consumer confidence. Contaminated exports can lead to border rejections and reputational damage for producers, particularly in low- and middle-income countries.

Table 1. Sources of Microbial Contamination in the Food Chain

Source Category	**Type of Microbial Contaminants**	**Examples**
Agricultural Inputs	Bacteria, Fungi, Viruses, and Parasites	Use of untreated wastewater, contaminated soil, animal feces, organic fertilizers, and pesticides
Animal Husbandry	Zoonotic Pathogens (e.g., *Salmonella*, *E. coli*)	Infected livestock, cross-contamination during milking or slaughter
Harvesting Practices	Bacteria, and Spoilage Microorganisms	Poor hygiene of harvest tools and hands, dirty containers
Processing Facilities	Listeria, *Clostridium*, Yeasts, and Molds	Poor sanitation, biofilms in equipment, condensation, and temperature abuse
Water Supply	Enteric Pathogens, and Protozoa	Use of contaminated water in washing, cooling, or processing
Storage and Packaging	Molds, and Spoilage Bacteria	Improper refrigeration, inadequate packaging, and damaged containers

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Table 1. Continued

Source Category	Type of Microbial Contaminants	Examples
Food Transportation	Bacteria (e.g., *Listeria monocytogenes*)	Cross-contamination from dirty trucks or mixed loads
Retail and Markets	Enteric Bacteria, Viruses	Exposure to open air, handling by multiple individuals, and unhygienic surfaces
Consumer Handling	*Campylobacter*, *E. coli*, Norovirus	Inadequate cooking, improper storage, and poor kitchen hygiene

2.2. Microorganisms of Concern

The microorganisms implicated in foodborne illnesses include a wide array of bacteria, viruses, parasites, and fungi. Bacterial pathogens such as *Salmonella, Escherichia coli* O157:H7, *Campylobacter, and Listeria monocytogenes* are particularly notable due to their association with severe health outcomes (El-Khishin et al., 2017; Teklemariam et al., 2023). Table 2 presents some of the most common foodborne microbes, their typical sources, and the health effects they may cause. This information provides a foundation for developing targeted surveillance, preventive strategies, and response mechanisms in food safety management. These pathogens induce gastrointestinal distress and can lead to more serious complications, such as kidney failure and meningitis, underscoring the importance of stringent food safety measures throughout the food supply chain (El-Khishin et al., 2017; Rabiee et al., 2024).

Viruses such as norovirus and hepatitis A are primarily transmitted through contaminated water and food, highlighting another aspect of microbial contamination (Casado et al., 2024). Parasites such as *Cryptosporidium* and *Giardia* can be introduced into the food chain through animal feces, often through contaminated water sources used for irrigation or cleaning (Ravaliya et al., 2014). Also, some fungi, particularly molds that produce mycotoxins, can contaminate grains and other agricultural products, posing additional health risks during consumption (Casado et al., 2024).

Table 2. Common microbes that contaminate food and food products

Microorganism	Type	Commonly Contaminated Foods	Health Effects / Diseases
Salmonella spp.	Bacteria	Poultry, eggs, meat, dairy, and vegetables	Salmonellosis (fever, diarrhea, abdominal cramps)
Escherichia coli (E. *coli*) O157:H7	Bacteria	Undercooked beef, raw milk, and vegetables	Hemorrhagic colitis, Hemolytic Uremic Syndrome (HUS)
Listeria monocytogenes	Bacteria	Deli meats, unpasteurized cheese, smoked fish	Listeriosis (fever, meningitis, miscarriage in pregnancy)
Campylobacter jejuni	Bacteria	Raw poultry, milk, and untreated water	Gastroenteritis, Guillain-Barré syndrome
Clostridium botulinum	Bacteria (spore-forming)	Canned foods, fermented fish, and honey	Botulism (paralysis, respiratory failure)
Staphylococcus aureus	Bacteria	Improperly stored meats, dairy, and pastries	Food poisoning (nausea, vomiting, abdominal cramps)
Norovirus	Virus	Shellfish, salads, contaminated water	Viral gastroenteritis (diarrhea, vomiting)
Hepatitis A virus	Virus	Raw shellfish, fresh produce, and infected handlers	Hepatitis A (liver inflammation, jaundice)
Toxoplasma gondii	Parasite	Undercooked pork, contaminated soil	Toxoplasmosis (flu-like symptoms, risk in pregnancy)
Cryptosporidium parvum	Parasite	Contaminated water, unwashed produce	Cryptosporidiosis (watery diarrhea, stomach cramps)
Aspergillus flavus	Fungi (Mold)	Grains, nuts (e.g., peanuts, maize)	Aflatoxicosis (liver damage, carcinogenic effects)
Penicillium spp.	Fungi (Mold)	Spoiled fruits, bread, and cheeses	Mycotoxicosis (allergic reactions, possible toxicity)
Bacillus cereus	Bacteria (spore-forming)	Cooked rice, pasta, and sauces	Food poisoning (emetic and diarrheal syndromes)

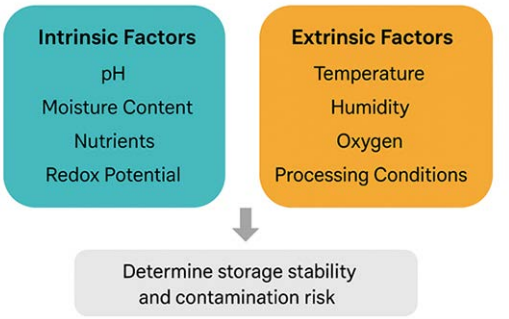
2.3. Factors Influencing Microbial Presence and Growth

Multiple factors influence the presence and growth of microorganisms in the food environment. These include intrinsic factors such as pH, moisture content, temperature, and nutrient availability, as well as extrinsic factors like the surrounding environment, processing methods, and storage conditions (Karuppuchamy et al., 2024). For instance, the moisture content of food is a crucial determinant of microbial growth; high-moisture foods, such as dairy and meat products, are particularly conducive to the proliferation of pathogens (Karuppuchamy et al., 2024). Conversely, low-moisture foods can harbor pathogens that survive longer under

these conditions, complicating control measures (Finn et al., 2013; Chime et al., 2016; Karuppuchamy et al., 2024).

Temperature also plays a significant role, as different microorganisms thrive at varying temperatures. The "danger zone" (between 40°F and 140°F) is particularly critical, as it is where bacteria multiply rapidly. Therefore, maintaining proper temperature control during storage, processing, and preparation is essential for minimizing microbial risks (Karuppuchamy et al., 2024; Rabiee et al., 2024). Climate change is increasingly recognized as a factor that alters microbial ecology in the food chain. Rising temperatures, extreme weather events, and water scarcity may exacerbate contamination risks by affecting pathogen survival and distribution in soil, water, and animal reservoirs. While antimicrobial agents are widely used to enhance food safety, their misuse or overuse may contribute to the emergence of antimicrobial-resistant strains, posing long-term threats to food systems. (Murphy et al., 2018; Teklemariam et al., 2023). Environmental conditions such as humidity and the presence of competing microbial flora can influence microbial populations in food (Ogwu et al., 2013; Osawaru et al., 2013; Ogwu and Osawaru, 2014; Oliveira et al., 2020). Cross-contamination, which occurs when pathogens are transferred from one food or surface to another, can significantly raise the microbial burden on food products. Mechanisms such as lapses in cleaning protocols and improper food handling contribute to cross-contamination risks (Karanth et al., 2023). Socioeconomic disparities in food handling infrastructure, sanitation, and public awareness also contribute to microbial contamination risks, especially in low-resource environments. Inadequate training among food handlers and limited access to cold chain logistics increase the vulnerability of certain populations to foodborne illnesses.

Figure 2. Intrinsic and extrinsic factors affecting microbial growth in food products



3.0. MICROBES IN FOOD: SAMPLING STRATEGIES AND SAMPLE PREPARATION

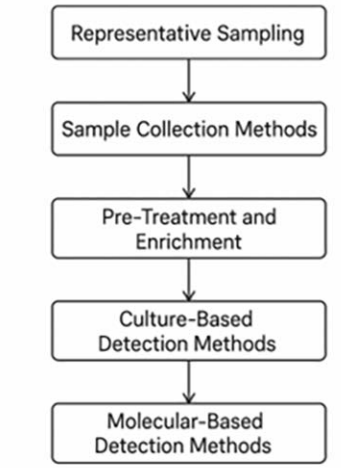
Microbial contamination of food poses significant public health risks, underscoring the crucial need for representative sampling strategies, effective sample collection methods, and pretreatment and enrichment procedures. These components are crucial for detecting the presence and concentration of foodborne pathogens in various food sources. Figure 2 outlines the sequential steps involved in detecting microbial contaminants in food products. The process begins with representative sampling to ensure that the food sample accurately reflects the batch or environment. Following this, appropriate sample collection methods are employed to maintain the specimen's integrity. Pre-treatment and enrichment steps are then used to enhance the growth or detectability of target microorganisms. Detection is achieved through culture-based techniques for isolation and identification, followed by molecular-based methods for confirmation and high-sensitivity diagnostics. This integrated workflow is critical for ensuring food safety and protecting public health.

3.1. Principles of Representative Sampling

Adequate representative sampling in food microbiology is crucial for ensuring the accurate detection and quantification of pathogens and spoilage microorganisms. The principles of representative sampling determine that samples must accurately reflect the entire batch of food being tested. This is crucial given the heterogeneity of microbial distribution within food matrices, which can vary significantly due to factors such as food type, surface area, and processing methods (Scallan et al., 2011; Cauteren et al., 2017; Pakbin et al., 2023). Historically, non-representative samples have led to misleading results, hindering efforts to assess the risk posed by pathogens present in food accurately (Scallan et al., 2011).

Furthermore, the selection of sampling techniques should consider the size and nature of the food items being sampled. For solid foods, such as meats, fruits, and vegetables, sampling should be systematic and involve specific units to allow for more accurate extrapolation of results to batches. In contrast, for liquids and semi-solid products, a methodical composite sampling approach should be employed, capturing variability across different container locations (Lanzas et al., 2011; Tao et al., 2022). Notably, larger sample sizes generally increase the reliability of the results, demonstrating that a balance must be struck between practicality and statistical validity (Troja et al., 2024).

Figure 3. Workflow for Detection of Microbial Contaminants in Food Samples



3.2. Sample Collection Methods

The detection of microbial contaminants in food products requires a systematic approach to ensure accuracy and reliability. Figure 2 outlines the essential steps in the detection pipeline, starting from representative sampling and progressing through sample collection, enrichment, and both culture-based and molecular-based detection methods. Each step is critical to ensuring the integrity of the results and the safety of food products. Sample collection methods differ between solid, liquid, and swab samples, each presenting unique challenges and considerations. For solid food samples, aseptic techniques must be employed, wherein food matrices are excised and aggressively mixed to form a composite sample (Jokerst et al., 2012; Amalaradjou and Bhunia, 2012). For instance, comminuted products, such as ground meat, require mixing from various parts of the batch to ensure that bacterial populations are accurately represented. Liquid samples, particularly those from beverages or processed foods, require mixing before collection to prevent bacterial stratification caused by density differences (Neff et al., 2019).

Swabs serve as a critical method for sampling surfaces and equipment that come into contact with food, particularly in bacteriological studies designed to assess hygiene practices in food establishments (Zhang et al., 2023; Kant et al., 2018). The efficacy of swabbing relies significantly on the technique's adherence to protocols, such as consistently wiping surfaces in a specified geometric pattern to maximize the recovery of microorganisms (Sriramulu, 2024). The choice of swabbing material can

also impact the results, as materials such as premoistened swabs are preferred over dry applications, which may inhibit microbial recovery (Pava-Ripoll et al., 2015).

3.3. Pre-Treatment and Enrichment Procedures

Once samples are collected, pretreatment and enrichment processes are paramount for isolating pathogens from the food matrix. Pre-treatment typically involves homogenization, where samples are blended to facilitate the more efficient extraction of microorganisms (Kim et al., 2021; Wei and Zhao, 2018). This step is crucial, as many pathogens exist within biofilms or as viable but non-culturable forms, both of which can evade detection unless properly processed (Zhang et al., 2021). For example, culturing foodborne pathogens in laboratory settings often requires enrichment to increase the pathogen load to detectable levels (Xiao et al., 2022).

Enrichment involves incubation in selective media that favors the growth of specific pathogens while inhibiting the growth of non-target organisms. This methodology is vital for detecting organisms such as Salmonella or Listeria, which can be outcompeted by the dominant microbiota present in food samples (Vanegas et al., 2017). Techniques such as PCR and next-generation sequencing facilitate the identification process, enabling researchers to overcome common limitations associated with classical culturing techniques by directly amplifying nucleic acids from target pathogens (Lee et al., 2023).

Importantly, microbial isolation and identification methods must adapt to emerging challenges such as antibiotic resistance. The application of rapid sequencing techniques and metagenomics enables the comprehensive characterization of microbial communities and the detection of resistance genes within food, thereby enhancing the efficacy of surveillance and control strategies (Beuchat et al., 2013). Implementing these strategies not only facilitates accurate pathogen identification but also informs public health interventions aimed at reducing the incidence of foodborne illnesses. Given the complexities of food matrices and microbial interactions, continuous methodological advancements and adherence to best practices in sampling and detection are essential to safeguard food safety globally.

4.0. CULTURE-BASED DETECTION METHODS FOR MICROBES IN FOOD

Culture-based detection methods remain foundational in food microbiology due to their reliability, simplicity, and ability to isolate viable pathogens (Izah et al., 2024). These methods involve the use of selective and differential media tailored to support the growth of target microorganisms while inhibiting the growth of other

microorganisms. Incubation conditions such as temperature, time, and atmospheric environment are optimized for each microbial group (Mahunu et al., 2024). Table 3 summarizes commonly used culture media, their incubation parameters, and typical applications in food safety monitoring, highlighting their continued relevance in identifying microbial contaminants in various food matrices.

Table 3. Culture Media and Conditions for Identifying Foodborne Microorganisms

Microorganism	Selective/Differential Media	Incubation Conditions	Application in Food Testing
Escherichia coli (*E. coli*)	Eosin Methylene Blue (EMB), MacConkey	37°C, 24 hours, aerobic	Detection in raw meats, dairy, and water
Salmonella spp.	Xylose Lysine Deoxycholate (XLD), SS agar	35–37°C, 24–48 hours, aerobic	Poultry, eggs, dairy, and ready-to-eat products
Listeria monocytogenes	PALCAM, Oxford agar	30°C, 24–48 hours, microaerophilic	Ready-to-eat meats, soft cheeses, and produce
Staphylococcus aureus	Baird-Parker agar	35–37°C, 24–48 hours, aerobic	Dairy products, cooked meats, and bakery items
Clostridium perfringens	Tryptose Sulfite Cycloserine (TSC)	45°C, 24 hours, anaerobic	Cooked meats, gravies, and stews
Campylobacter jejuni	Campy-BAP, Skirrow agar	42°C, 48 hours, microaerophilic	Poultry, raw milk, and water
Bacillus cereus	Mannitol Egg Yolk Polymyxin (MYP)	30°C, 24 hours, aerobic	Rice dishes, dairy, and processed foods
Vibrio spp.	Thiosulfate Citrate Bile Salts Sucrose (TCBS)	35°C, 18–24 hours, aerobic	Seafood, especially raw shellfish
Yeasts and Molds	Potato Dextrose Agar (PDA), DRBC agar	25–28°C, 3–5 days, aerobic	Fruits, baked goods, dairy, and fermented products
Shigella spp.	Hektoen Enteric (HE) agar	35–37°C, 24 hours, aerobic	Leafy greens, salads, and water

Culture-based detection methods, including traditional plate counts and the Most Probable Number (MPN) technique, remain essential in food microbiology despite advancements in molecular techniques. Each method has its advantages and limitations, which shape its applicability in food safety monitoring and microbial ecology studies. Traditional plate counts involve isolating microorganisms from food samples on selective agar media, followed by incubation and counting the colonies. This method is considered the 'gold standard' for microbial detection due to its ability to identify viable bacteria, especially in complex food matrices (Schultz et al., 2012; Rohde et al., 2015; Kim and Kim, 2020). While straightforward, plate counts can be time-consuming and labor-intensive, typically requiring 24 to 72 hours for results (Schultz et al., 2012; Margot et al., 2013). Additionally, this technique may over-

look significant pathogens classified as viable but non-culturable (VBNC), leading to underestimations of microbial counts (Kim and Kim, 2020; Yang et al., 2020).

The MPN technique is a statistical method used to estimate the concentration of viable microorganisms in a sample by performing serial dilutions and subsequent incubation to evaluate growth (Line et al., 2011; Crowley et al., 2010). Also, MPN has been enhanced by automated systems, such as TEMPO, allowing for rapid and reliable quantification of microorganisms, like *Escherichia coli*, in various food products (Katase and Tsumura, 2011; Lee et al., 2021). Automated MPN reduces labor and minimizes human error associated with manual dilutions and counting, making it particularly attractive in high-throughput laboratory settings (Line et al., 2011). However, like traditional culture methods, the MPN technique is not immune to the challenges posed by VBNC microorganisms, which can skew results (Yang et al., 2020).

Both traditional plating and MPN techniques have demonstrated effectiveness in detecting key foodborne pathogens such as *Salmonella spp.* and *E. coli* in diverse food matrices, including poultry and dairy products (Schultz et al., 2012; Margot et al., 2013). These methods also facilitate the investigation of epidemiological trends and microbial load assessments, which are critical in food safety protocols. However, traditional approaches have limitations regarding the time required for analysis and the dependency on skilled personnel for interpretation (Afshari et al., 2012; Shangguan et al., 2023).

Despite significant improvements in sensitivity and speed, molecular methods like PCR and isothermal amplification are often employed in tandem with traditional techniques to enhance detection capabilities (Tongphrom et al., 2018; Yordanova and Andonova, 2024). For instance, combining MPN with PCR can enhance sensitivity and specificity, resulting in improved outcomes in detecting foodborne pathogens during routine quality control measures (Crowley et al., 2010; Lee et al., 2021). While these molecular techniques have advanced the field, they may come with higher costs and often require specialized training, making them less accessible to specific laboratories, particularly in resource-limited settings (Yordanova and Andonova, 2024; Schultz et al., 2012).

Furthermore, alternative detection methods such as loop-mediated isothermal amplification (LAMP) offer potential improvements in pathogen detection in food products. Also, LAMP is known for its rapidity and sensitivity, capable of detecting pathogens in as little as 40 minutes (Yang et al., 2016). This trend toward molecular techniques reflects the growing demand for faster analytical methods in response to the rising incidence of foodborne illnesses, where timely detection is crucial (Tongphrom et al., 2018). In reconciling these methodologies, food safety laboratories must adopt a multipronged approach that incorporates both traditional culture-based methods and rapid molecular techniques. Understanding the strengths

and limitations of each approach enables practitioners to design testing protocols that optimize microbial detection while conserving resources and time (Margot et al., 2013; Yang et al., 2020). Moreover, integrating cultural and molecular methods can provide a comprehensive view of microbial populations and dynamics within food environments, particularly in understanding the development of antibiotic resistance in foodborne pathogens (McLain et al., 2016; Rohde et al., 2015).

5.0. IMMUNOLOGICAL DETECTION TECHNIQUES FOR MICROBES IN FOOD

Immunological methods are indispensable tools in food safety management, enabling the sensitive and specific detection of microbial pathogens. These techniques leverage antigen-antibody interactions to identify contaminants with high accuracy, even in complex food matrices.

5.1. Enzyme-linked immunosorbent assay (ELISA)

Among immunoassays, ELISA stands out for its sensitivity, specificity, and capability for high-throughput analysis. ELISA is commonly employed to detect a range of foodborne pathogens, including *Salmonella* spp. and *Escherichia coli* O157:H7 (Zhao et al., 2014; Law et al., 2015; Park et al., 2018). This technique relies on antigen-antibody interactions, whereby specific antibodies capture the target pathogen from food matrices, followed by a colorimetric or fluorescent signal that indicates the presence of the pathogen. For example, modified versions of ELISA have demonstrated efficacy in detecting pathogens in various complex food samples, meeting the critical criteria necessary for robust food safety monitoring (Zheng et al., 2019; Vargas et al., 2018).

5.2. Lateral flow devices and dipstick assays

Lateral flow devices and dipstick tests represent another category of immunological assays that offer rapid and user-friendly detection of pathogens in food samples. These point-of-care tests are characterized by their simplicity and speed, providing results within minutes without the need for elaborate laboratory setups. The functionality of these devices hinges on a similar antigen-antibody interaction found in ELISA; however, the format is designed to yield visual results through color changes or lines on a test strip. Although lateral flow assays excel in rapid diagnostics, their sensitivity can sometimes fall short compared to more elaborate

methodologies such as ELISA, particularly in detecting low concentrations of pathogens (Park et al., 2020).

5.3. Immunomagnetic separation (IMS)

IMS is a powerful technique that utilizes magnetic beads coated with specific antibodies to isolate and concentrate target pathogens from complex mixtures commonly found in food matrices. This method enhances the detection sensitivity of pathogens such as Salmonella and E. coli and helps mitigate interference from non-target microorganisms typically present in food samples (Hsu et al., 2014; Wei et al., 2016). The application of IMS has shown promise in improving the efficiency of pathogen recovery during food analysis. For instance, the combination of IMS with molecular techniques, such as multiplex PCR (mPCR), has been utilized to streamline the detection process for multiple pathogens simultaneously. This integration facilitates thorough pathogen analysis in various food samples, ranging from poultry to dairy products, effectively reducing the time to obtain results and addressing regulatory demands and public health concerns (Castellví et al., 2010; Yoshitomi et al., 2012).

5.4. Emerging technologies and hybrid systems

The complexity of food matrices often hinders the accurate detection of pathogens due to the presence of inhibitors that affect both traditional culturing methods and PCR amplifications. One significant advantage of immunomagnetic separation is its ability to minimize these inhibitory effects, thus enhancing the recovery rates of foodborne pathogens. For example, when applied to dairy products, IMS has been demonstrated to significantly improve the detection of Staphylococcus aureus from food matrices, thereby increasing the reliability of pathogen monitoring in these environments (Wei et al., 2016). Moreover, the use of rapid protocols involving IMS has become increasingly relevant. As identified in studies, methods such as real-time PCR combined with IMS enable the rapid enumeration of pathogens, including *E. coli* O157:H7, highlighting the efficacy of this approach in food safety surveillance (Onmaz et al., 2013).

In addition to conventional methods, innovative advances in nanotechnology and biosensor development are expanding the landscape of immunological detection techniques. These advancements can significantly enhance sensitivity and specificity. For instance, the integration of nanoparticles in biosensors for pathogen detection is fostering novel platforms that allow rapid and accurate quantification of pathogens in various food environments. Researchers are increasingly utilizing magnetic nanoparticles to develop highly sensitive electrochemical biosensors,

which, when combined with immunomagnetic separation, provide a powerful tool for the real-time detection and monitoring of foodborne pathogens (Wang et al., 2017). Such techniques improve the response times in identifying contamination, effectively addressing public health concerns, and ensuring food safety compliance.

Moreover, advanced spectroscopic methods, such as Fourier-transform infrared (FT-IR) spectroscopy, are being explored in conjunction with immunomagnetic separation to provide a multidimensional approach for detecting foodborne pathogens. Target pathogens can be concentrated using IMS, and FT-IR can then analyze spectral data to differentiate between various pathogen serovars effectively. This approach significantly reduces analysis times and enhances the specificity of microbial identification, demonstrating the versatility and efficacy of combining immunological techniques with advanced analytical methodologies in improving food safety (Castellví et al., 2010).

5.5. Challenges and outlook

Despite the significant progress in immunological detection techniques, challenges such as the search for novel antibodies and improving detection limits persist. Continuous research is crucial for developing antibodies that exhibit high specificity and affinity toward target pathogens, particularly as new strains of bacteria emerge. Furthermore, improving the detection limits of immunoassays remains critical for ensuring the safety of food products that may contain low levels of bacterial pathogens, which is common in various food processing environments (Foddai et al., 2010; Amoako et al., 2012; Li et al., 2010). The evolution of these techniques suggests a broader trend of utilizing integrative approaches, combining various detection modalities, for a more comprehensive assessment and management of foodborne pathogens.

I propose this table: Table 4 summarizes key immunological methods used in food safety diagnostics, comparing their detection speed, sensitivity, instrumentation requirements, and optimal use cases. It highlights how each technique contributes to pathogen identification across various settings, from field-level screening to laboratory-based confirmatory analysis.

Table 4. Comparative overview of immunological detection techniques for foodborne pathogens

Technique	Time to result	Sensitivity	Instrumentation	Best use cases
ELISA	2-4 hours	High	Moderate	Routine lab diagnostics
Lateral flow assays	5-20 minutes	Moderate	Minimal	On-site screening
IMS + PCR	3-5 hours	Very High	High	Confirmatory, multi-pathogen
IMS + FT-IR	<2 hours	High	Advanced	Strain-level identification
Nanoparticle biosensor	< 1 hour	Very High	Specialized	Real-time monitoring

6.0. MOLECULAR AND NUCLEIC ACID-BASED METHODS OF DETECTING MICROBES IN FOOD

Molecular methods have transformed the detection and characterization of microbial pathogens in food by enabling high-throughput, culture-independent analysis (Figure 3). Techniques such as metagenomics, metatranscriptomics, and whole genome sequencing (WGS) offer comprehensive insights into microbial diversity, strain-level identification, and functional activity. These methods analyze DNA or RNA directly from food samples using targeted or shotgun sequencing approaches, followed by bioinformatic analyses. This integrated workflow enables the rapid and precise identification of pathogens, including unculturable or low-abundance organisms, thereby enhancing food safety surveillance and outbreak investigations. Conventional PCR, quantitative PCR (qPCR), isothermal methods like LAMP (loop-mediated isothermal amplification) and RPA (recombinase polymerase amplification), and digital PCR (dPCR) offer efficient means to identify foodborne pathogens.

Conventional PCR remains a commonly utilized technique for microbial detection. Different strategies have been employed to enhance the sensitivity of PCR methods, such as pre-enrichment steps before testing food samples to increase microbial yield (Radji et al., 2010). In studies comparing PCR with conventional culturing methods for *Listeria monocytogenes*, PCR has demonstrated an improved detection rate, resulting in a more comprehensive assessment of food safety (Khan et al., 2014; Furmančíková et al., 2022). Furthermore, the integration of genetic markers into PCR has enabled differentiation among closely related microbial species, thereby improving specificity and accuracy (Radji et al., 2010; Wang and Salazar, 2015). Owing to its robust protocols and proven reliability, conventional PCR continues

to be refined and effectively applied due to its established protocols and reliability in various food matrices.

Quantitative PCR (qPCR) is an advancement over conventional PCR, allowing for the quantitative analysis of microbial populations. The ability to measure the number of nucleic acids present in a sample not only expedites the detection process but also provides insights into the microbial load—information crucial for food safety evaluations (Law et al., 2015). Studies have shown that qPCR can yield results with higher sensitivity compared to traditional cultivation methods. For instance, when detecting Salmonella in food samples, qPCR demonstrated the ability to identify lower pathogen concentrations within a shorter turnaround time compared to classical methods (Furutani et al., 2016).

Isothermal amplification techniques, such as LAMP and RPA, are emerging as powerful alternatives that circumvent the temperature cycling step associated with traditional PCR. These methods are exceptionally robust, providing rapid and efficient amplification of target nucleic acids at a constant temperature. LAMP has displayed impressive sensitivity and specificity in detecting foodborne pathogens, making it particularly suitable for on-site testing in food production environments (Foddai and Grant, 2020). The advantages of isothermal amplification techniques include a reduced need for sophisticated thermal cyclers and shorter assay times, which are critical for quick screening in public health settings (Law et al., 2015).

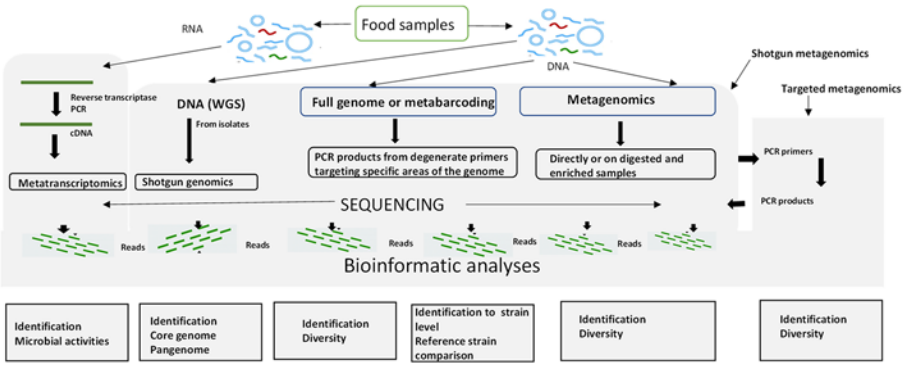
Digital PCR (dPCR) enables the absolute quantification of nucleic acids, eliminating the need for standard curves. This technique's high precision makes it suitable for applications such as monitoring foodborne pathogens at very low abundance (Wang et al., 2018). The dPCR approach is noted for its ability to detect rare target sequences in complex samples, thereby increasing confidence in results compared to quantitative methods that may suffer from amplification biases (Mangal et al., 2015). While dPCR is still gaining traction in food safety, its unique advantages position it well for future developments in enhancing microbial safety regulations.

The integration of molecular techniques into food safety protocols extends beyond traditional methods. The development of biosensors and rapid detection assays utilizing nucleic acid amplification methods offers the potential for real-time monitoring (Zeng et al., 2016). Advances in microarray technologies and metagenomics enable the concurrent assessment of multiple pathogens in a single assay, addressing the complexity of food matrices without needing labor-intensive culturing steps (Li et al., 2017). These innovations reflect ongoing efforts to enhance the capability and speed of microbial detection methods.

Hybridization techniques, such as fluorescence in situ hybridization (FISH), complement molecular detection methods by providing visualization and confirmation of pathogens directly in food samples (Rocha et al., 2019; Rohde et al., 2015). FISH applications have demonstrated promising results in terms of specificity and

reliability in pathogen detection, offering visual confirmation that is often lacking in conventional molecular diagnostics. The combination of nucleic acid amplification and hybridization techniques embodies a comprehensive approach to microbial detection, maximizing accuracy while minimizing time-to-result.

Figure 4. Molecular Approaches for Microbial Pathogen Detection in Food: Genomics, Metagenomics, and Transcriptomics



Source: *Aladhadh (2023)*

7.0. ADVANCED TECHNIQUES FOR DETECTING MICROBIAL CONTAMINANTS OF FOOD: HIGH-THROUGHPUT AND METAGENOMICS

The detection of microbial contaminants in food has undergone significant advancements through the application of advanced techniques, including high-throughput sequencing and metagenomics. This progression is primarily defined by two prominent methodologies: genetic (i.e., 16S rRNA gene and it's like) sequencing and shotgun metagenomics, both of which bring unique advantages and challenges to microbial profiling in food safety.

The 16S rRNA gene sequencing method primarily focuses on specific hypervariable regions of the 16S rRNA gene, which play a critical role in identifying and characterizing bacterial communities. A study by Chen et al. (2019) demonstrated that different primer pairs targeting various hypervariable regions in the 16S rRNA gene can yield distinct microbial profiles, emphasizing the importance of primer selection in obtaining accurate representations of microbial community composition. Moreover, this study indicated that amplicon-based techniques, despite their cost-effectiveness, often produce biased results primarily due to PCR amplification

discrepancies, which could overlook low-abundance taxa critical for understanding foodborne pathogen dynamics (Chen et al., 2019; Zaheer et al., 2018). In addition to 16S rRNA and shotgun metagenomics, internal transcribed spacer (ITS) and 18S rRNA gene sequencing have emerged as critical tools for detecting fungal and eukaryotic microbial contaminants in food. ITS sequencing, often referred to as the "universal fungal barcode," is widely used for profiling fungal communities due to its high taxonomic resolution at the species level, making it ideal for identifying spoilage fungi and mycotoxin-producing species (Schoch et al., 2012). Similarly, 18S rRNA sequencing enables the comprehensive detection of eukaryotic microorganisms, including protozoa and microscopic parasites, that bacterial-focused approaches may overlook. When used in combination with high-throughput platforms, ITS and 18S sequencing expand the scope of metagenomic surveillance to include a broader spectrum of microbial threats in food systems, thereby enhancing the accuracy and inclusivity of food safety assessments (Nilsson et al., 2019; Pawlowska et al., 2020).

Conversely, shotgun metagenomics allows for a more comprehensive approach by sequencing the entire pool of genetic material in a sample. This technique not only enhances the retrieval of microbial diversity but also facilitates the identification of non-culturable organisms that may pose significant risks in food products. Research by Ge et al. (2025) suggests that shotgun metagenomics and 16S rRNA sequencing serve different purposes, with the former being particularly effective for gaining insights into the overall microbiome composition, especially in low-complexity environments such as food. Furthermore, the application of shotgun sequencing in the study of complex ecosystems, such as those found in animal food microbiomes, has expanded our understanding of microbial interactions and resistomes (Ge et al., 2025; Jagadeesan et al., 2019).

One critical advantage of shotgun metagenomics is its capacity to provide strain-level resolution and functional insights into microbial communities (Yulandi et al., 2020; Buytaers et al., 2022). For instance, Yulandi et al. (2020) demonstrated that shotgun metagenomic analysis can capture a higher diversity of microbial taxa compared to conventional 16S rRNA methods, particularly in samples such as tempeh. This improved accuracy is vital for tracing contamination sources during outbreak investigations and facilitating rapid food safety assessments (Grützke et al., 2019; Yulandi et al., 2020). However, while shotgun sequencing offers more in-depth analysis, it also presents challenges regarding the complexity of data interpretation and bioinformatics requirements (Sala et al., 2020; Buytaers et al., 2022).

The integration of bioinformatics tools plays a crucial role in managing the vast amount of data generated from both sequencing approaches. Effective interpretation of this data allows for accurate characterization of microbial communities and the detection of potential pathogenic organisms within food matrices. Advanced computational frameworks, such as PICRUSt2 and Tax4Fun2, are employed to infer

functional profiles based on 16S rRNA sequencing data, complementing the insights gained from shotgun metagenomics (Matchado et al., 2023). The prioritization of quantitative data and the coupling of various sequencing methods can enhance the robustness of microbial surveillance studies, ultimately informing public health responses and food safety strategies.

Despite the numerous advantages attributed to high-throughput methods, there are significant considerations surrounding methodological validity and sample processing. The risk of contamination during sample collection and processing remains a considerable challenge. Cumpănaş et al. (2020) highlighted the susceptibility of metagenomic techniques to contain contaminant DNA from environmental or procedural sources. This risk necessitates stringent quality control measures and standardized methodologies to ensure reliability of results across laboratories (Sala et al., 2020).

In the context of food safety, the application of shotgun metagenomics and 16S rRNA sequencing methods has illustrated their respective utility in identifying and profiling microbial pathogens (Grützke et al., 2019; Koutsoumanis et al., 2019). Various studies have noted the complementary role these techniques can play in food microbiology, with shotgun metagenomics being particularly advantageous for recognizing threats from pathogens that traditional culture-based methods may fail to detect (Chen et al., 2021; Jagadeesan et al., 2019). The significance of utilizing both approaches lies in their ability to form a comprehensive overview of microbial dynamics, thereby informing risk assessment and control measures in food production environments. Furthermore, evolving concepts surrounding the microbial resistome highlight the importance of surveillance in microbial communities. Zaheer et al. (2018) indicated that sequencing depth significantly influences the ability to characterize microbial and resistome diversity, reinforcing the notion that comprehensive metagenomic mapping can inform effective strategies for combating antibiotic resistance in food contaminants. As the field advances, integrating these advanced techniques into standardized food safety protocols is critical for maintaining public health standards.

While traditional microbiological methods still play a vital role in detecting food pathogens, modern sequencing techniques, including shotgun metagenomics and 16S rRNA gene sequencing, are invaluable tools that facilitate more sensitive and comprehensive microbial analysis. These methodologies, paired with rigorous bioinformatics and contamination risk assessments, can significantly bolster food safety across production chains (Bogatyrev and Ismagilov, 2020; Grützke et al., 2019). As the knowledge base surrounding microbial ecology expands, there is an imperative to adopt innovative technologies that enhance our capacity to safeguard food chain integrity.

8.0. BIOSENSORS AND LAB-ON-A-CHIP APPROACHES FOR DETECTING MICROBIAL CONTAMINANTS IN FOOD

Biosensors and lab-on-a-chip (LOC) technologies have revolutionized the detection of microbial contaminants in food, providing innovative and efficient methods that significantly enhance food safety. This response focuses on the various types of biosensors and microfluidic platforms, as well as their integration with real-time monitoring systems, thereby providing a comprehensive overview of the current advancements and challenges within this field.

8.1. Types of Biosensors

Biosensors are analytical devices that convert biological responses into electrical signals, allowing for the detection of various analytes, including microbial contaminants. The two primary types of biosensors utilized for food safety are electrochemical and optical biosensors. Electrochemical biosensors are particularly well-regarded for their sensitivity, selectivity, speed, and cost-effectiveness. They often employ electrochemical techniques such as amperometry and impedance spectroscopy to detect low concentrations of pathogens in complex food matrices, making them suitable for applications in food analysis (Curulli, 2021; Wang et al., 2022; Majer-Baranyi et al., 2023).

The integration of nanomaterials into electrochemical biosensors has further enhanced their performance. Nanomaterials, such as carbon nanotubes and metal nanoparticles, offer an increased surface area and enhance electrochemical interactions between the electrode surface and target analytes. This amplification of the biosensing signal is critical for achieving low detection limits in food safety applications (Curulli, 2021; Raj et al., 2021). The growing body of research demonstrates that electrochemical biosensors can be tailored for specific pathogens, such as Salmonella, by using selective bioreceptors like antibodies, aptamers, or bacteriophages embedded in their designs (Awang et al., 2021; Rizzotto et al., 2023; Zhou et al., 2024)

On the other hand, optical biosensors rely on the transduction of light-based signals, which can also detect microbial contaminants. They often utilize techniques such as surface plasmon resonance (SPR), fluorescence resonance energy transfer (FRET), and reflectance measurements to monitor interactions at the molecular level (Duan et al., 2016). Despite their advantages in specificity and sensitivity, optical biosensors tend to be more expensive and technically demanding than their electrochemical counterparts (Vidić et al., 2019).

8.2. Microfluidic Platforms

Microfluidics represents another transformative technology in food safety, enabling the miniaturization of laboratory processes and allowing for the simultaneous processing of multiple samples in a minimal volume. Microfluidic devices are characterized by their ability to manipulate fluids at the microscale, typically within channels with diameters of less than 1 mm. These devices can integrate various analytical processes, including sample extraction, reaction, and detection, all on a single chip, which simplifies the workflow and significantly reduces analysis time. Nasseri et al., 2018; Parihar et al., 2023).

Recent advancements in microfluidic biosensors have focused on the rapid detection of foodborne pathogens. For instance, antibody-coated microspheres are utilized in microfluidics to increase the capture efficiency of pathogen detection through antigen-antibody interactions (Song et al., 2020). The miniaturization capabilities of microfluidics enable their deployment in point-of-care (POC) testing scenarios, significantly enhancing the accessibility of food safety diagnostics, particularly in resource-limited regions (Li et al., 2015; Nasseri et al., 2018; Zhou et al., 2024).

Moreover, the integration of microfluidic platforms with electrochemical biosensing allows for enhanced detection sensitivity while maintaining rapid analysis capabilities. Such a combination enables the development of portable, low-cost, and easy-to-use devices that are well-suited for use in food processing environments (Awang et al., 2021; Rizzotto et al., 2023). The automated nature of microfluidics reduces operator error and variability, resulting in more consistent detection of microbial contaminants.

8.3. Integration with Real-Time Monitoring

The seamless integration of biosensors with real-time monitoring systems is an essential feature that enhances food safety management. The ability to provide immediate feedback enables food safety professionals to respond promptly to contamination risks and prevent outbreaks. Innovative approaches, such as using the Internet of Things (IoT) to connect biosensing devices to cloud-based data analysis platforms, enable continuous monitoring of food products throughout the supply chain (Parihar et al., 2023; Wang et al., 2022; Mishra et al., 2018). With integrated real-time monitoring, biosensors can continuously assess the safety of food products throughout their processing, storage, and distribution. For instance, battery-operated electrochemical biosensors linked to mobile applications provide timely notifications about microbial contamination, which facilitates rapid response measures to mitigate health risks (Rizzotto et al., 2023; Vidić et al., 2019). These advances are crucial,

particularly as the food industry faces increasing challenges with food safety and public health (Mishra et al., 2018; Wang et al., 2022).

8.4. Other Considerations

Aside from technological advancements, several challenges impede the widespread adoption of biosensor and microfluidics technology in the food industry. These challenges include the need for robust calibration protocols, the variability in complex food matrices that can interfere with detection, and the regulatory framework governing food safety testing methods (Wijayanti et al., 2023; Curulli, 2021). Furthermore, while advancements in nanomaterials and biosensing techniques have shown great promise, concerns remain regarding the long-term stability and reproducibility of biosensors in real-world applications (Song et al., 2020; Raja et al., 2021; Pham et al., 2021).

9.0. CRISPR-BASED DETECTION SYSTEMS FOR DETECTING MICROBIAL CONTAMINANTS IN FOOD

The advent of CRISPR-based systems has revolutionized various fields, particularly the detection of microbial contaminants in food safety. Specific technologies such as the CRISPR-Cas12 and Cas13 systems, including platforms like SHERLOCK (Specific High-sensitivity Enzymatic Reporter UnLOCKing) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), are at the forefront of this advancement. These systems enable precise and rapid identification of pathogenic microorganisms, which is essential for ensuring food quality and safety in an era where foodborne illnesses pose significant public health challenges (Brandt and Barrangou, 2019; Shin et al., 2022; Sun et al., 2024).

The CRISPR-Cas12 and Cas13 systems provide innovative solutions for real-time detection of various microbial contaminants in food matrices. For instance, CRISPR-based biosensors utilize the inherent nuclease activity of these proteins to cleave target sequences, thus facilitating the creation of detectable signals upon the recognition of specific nucleic acids associated with pathogens (Chakraborty et al., 2022; Li et al., 2022; Xie et al., 2024). These platforms extend detection capabilities to a broader range of targets, encompassing bacteria, viruses, and toxins, while optimizing the speed and sensitivity of existing methodologies. This is particularly useful in field settings where conditions may be less controlled, necessitating solutions that can perform effectively outside traditional laboratory environments (Shin et al., 2022; Wang et al., 2024).

One of the strengths of CRISPR-based detection systems lies in their adaptability and specificity. These systems can be engineered to target genomic sequences associated with foodborne pathogens, allowing for low detection limits and high accuracy (Xiong et al., 2019; Li et al., 2022). The versatility of CRISPR supports diverse signal readout mechanisms, including fluorescent, colorimetric, and electrochemical methods. This diversity not only enhances user-friendliness but also empowers non-specialists to conduct reliable food safety assessments (Sun et al., 2024; Xin et al., 2025). Notably, recent developments have integrated these CRISPR technologies with isothermal amplification protocols, thus improving sensitivity and practicality in various contexts (Sun et al., 2020).

However, the application and performance of CRISPR-based systems for food safety detection come with several technical considerations. Key challenges include potential off-target effects that may lead to false positives and the need for a reliable sample preparation process to ensure testing accuracy (Cui et al., 2024; Gootenberg et al., 2017). Standardization of CRISPR-based testing methods is crucial to facilitate their acceptance and implementation in regulatory frameworks (Li et al., 2023; Chen, 2024). Researchers emphasize the importance of developing user-friendly platforms that do not require extensive training and can be rapidly deployed in response to food safety threats (Brandt and Barrangou, 2019; Li et al., 2022).

The modularity of CRISPR systems enables researchers to tailor detection methods to the target of interest. Successful applications have been reported in identifying pathogens such as Salmonella, E. coli, and even viruses like Zika and Dengue within food samples (Zhang et al., 2022; Wang et al., 2024). Moreover, recent studies indicate that CRISPR-based biosensors can be developed into portable devices, significantly enhancing their feasibility for on-site detection in food production environments (Chakraborty et al., 2022; Sun et al., 2024). This shift toward real-time analysis aligns with industry and regulatory demands for traceability and rapid responses to contamination incidents, thus safeguarding public health and consumer trust (Brandt and Barrangou, 2019; Li et al., 2022).

Beyond the technical and adaptability aspects, the economic implications of CRISPR-based detection platforms are significant. They offer cost-effective solutions compared to traditional laboratory methods, fundamentally transforming the economics of food safety testing. For instance, simple device designs can lead to lower production costs, making them accessible to producers in different regions (Shin et al., 2022; Li et al., 2023). This accessibility is crucial for developing regions where food quality assurance resources may be limited, promoting global health standards across food systems (Moller and Liang, 2017; Sun et al., 2024). CRISPR-Cas12 and Cas13-based detection systems represent the cutting edge of microbial contamination detection in food safety, merging precision with practicality. Their inherent adaptability enables them to deliver tailored solutions that meet specific

industry needs, yielding rapid and reliable results in diverse environments. As research progresses, these technologies must be scaled, standardized, and integrated into existing food safety protocols to realize their potential and protect public health fully (Brandt and Barrangou, 2019; Li et al., 2022; Zhang et al., 2022).

10.0. VALIDATION, STANDARDIZATION, AND APPLICATIONS IN FOOD SAFETY RISK MANAGEMENT

The effective detection of microbial contaminants in food systems hinges not only on the sensitivity and specificity of the methods employed but also on their validation, standardization, and integration into broader food safety management frameworks. A robust detection strategy ensures timely and accurate identification of hazards, enabling preventative and corrective actions that protect public health and preserve market confidence.

10.1. Performance Metrics and Method Validation

Core performance parameters—sensitivity, specificity, and limit of detection (LoD)—form the baseline for evaluating any microbial detection method. Sensitivity measures a method's ability to identify true positives, while specificity captures its accuracy in recognizing true negatives (López-Campos et al., 2020). These metrics are particularly critical for ensuring reliable results in complex food matrices, where microbial loads can be low and inhibitors abundant.

The LoD is essential in ready-to-eat foods or minimally processed products, where even small quantities of pathogens, such as *Listeria monocytogenes* or *Salmonella,* may be hazardous (Hoorfar et al., 2004). Quantitative PCR and other molecular platforms have shown strong potential for low LoD and rapid turnaround times, though matrix interference must be controlled through effective sample preparation and control inclusion. Method validation ensures that detection technologies meet the expectations of both industry and regulatory standards. ISO 16140, a cornerstone in food microbiological method validation, prescribes comparative studies against standard reference methods to evaluate performance criteria, including repeatability, reproducibility, and robustness (ISO, 2020). Similarly, AOAC International provides structured validation pathways—Performance Tested Methods (PTMs) and Official Methods of Analysis (OMAs)—to certify new technologies for practical and regulatory use.

10.2. Standardization and Quality Assurance Systems

Reliable microbial detection demands both internal and external quality assurance. Internally, laboratories must implement routine controls (both positive and negative), calibrate instruments, and utilize personnel who have undergone proficiency testing. For molecular methods, internal amplification controls and inhibition checks are essential to ensure valid negative results and avoid false interpretations (Schrader et al., 2012). Externally, proficiency testing (PT) and inter-laboratory comparisons support continuous quality monitoring. Accreditation under ISO/IEC 17025 requires participation in PT schemes and the maintenance of traceable and reproducible documentation (ILAC, 2017). Together, these practices foster confidence in analytical results and facilitate international trade, as well as compliance with food safety legislation.

10.3. Integration into Food Safety Risk Management Frameworks

Validated and standardized detection methods become most impactful when integrated into proactive food safety risk management systems. One of the principal applications is in Hazard Analysis and Critical Control Point (HACCP) programs, where microbial testing confirms the control of biological hazards at critical points in the production process. For example, routine swab testing at food contact surfaces using validated PCR or immunoassay kits can verify the effectiveness of sanitation and prevent cross-contamination.

Detection data also play a pivotal role in Quantitative Microbial Risk Assessment (QMRA) models. These models quantify the risk of infection or illness from specific pathogens under varying exposure scenarios, relying on accurate microbial counts to inform dose–response and exposure assessments (FAO/WHO, 2016). The integration of high-throughput and culture-independent detection methods, such as metagenomics, enables the evaluation of microbial community shifts that may indicate emerging risks or spoilage conditions.

Another critical application is in traceability and response to product recalls. Timely and validated detection enables the rapid identification of contamination sources and the containment of affected batches. Molecular typing and whole-genome sequencing (WGS) have been increasingly employed to link clinical and foodborne isolates, enhancing traceback accuracy and supporting regulatory enforcement (Jackson et al., 2016).

10.4. Toward Harmonization and Predictive Resilience

Ultimately, the convergence of validated detection methods, QA systems, and real-time data integration supports a predictive and resilient food safety system. As food supply chains become more globalized and climate change alters microbial ecology, harmonized detection frameworks that are flexible, accurate, and scalable are crucial. Collaborative efforts among laboratories, regulatory agencies, and industry stakeholders anchored in scientific validation will be key to sustaining food integrity in a rapidly changing world.

11.0. CHALLENGES AND FUTURE PERSPECTIVES

Despite significant advances in microbial detection technologies, several persistent and emerging challenges continue to affect the reliability, accessibility, and implementation of these tools in food safety systems. Addressing these challenges is essential to ensure that detection methods are not only scientifically sound but also globally equitable and adaptable to real-world contexts. These challenges include:

11.1. Low-Resource Settings

One of the most pressing issues is the limited capacity for microbial detection in low-resource settings, particularly in low- and middle-income countries. Infrastructure deficiencies, including unreliable electricity, a lack of cold chain logistics, and limited laboratory space, impede the use of advanced diagnostics such as PCR, ELISA, or NGS (Aladhadh 2023). Additionally, human resource constraints, such as the shortage of trained microbiologists and laboratory technicians, further reduce the feasibility of deploying complex methods in rural or decentralized food systems. Even where rapid diagnostic kits exist, costs associated with reagents, equipment maintenance, and licensing can render these tools inaccessible (Oladeji et al., 2024). These limitations can delay the detection of contamination, compromise outbreak response, and contribute to persistent food safety burdens in vulnerable populations (Ogwu and Ogunsola, 2024). To bridge this gap, innovations must prioritize affordability, durability, ease of use, and minimal reliance on cold storage or skilled personnel.

11.2. Matrix Effects and Diagnostic Uncertainty

Food matrices vary significantly in composition, containing fats, proteins, polysaccharides, and polyphenols that can inhibit detection reagents or interfere with signal readouts. These matrix effects are a common source of false negatives or

compromised sensitivity, particularly in PCR and biosensor-based assays (Schrader et al., 2012). Conversely, false positives may arise from cross-contamination, residual DNA from dead microorganisms, or non-target amplification, leading to unnecessary recalls or reputational damage. Even validated methods can struggle to maintain performance across diverse food products such as dairy, meat, and fermented goods. As such, robust pretreatment protocols (e.g., filtration, centrifugation, and enzymatic digestion) and internal controls are essential for ensuring diagnostic reliability. Furthermore, field-deployable kits often face the challenge of striking a balance between simplicity and accuracy, necessitating ongoing refinement for both laboratory and point-of-care contexts.

11.3. Innovations in Portable Devices and AI-Driven Detection

Recent years have witnessed a surge in portable and point-of-need detection devices, offering promise for rapid microbial monitoring in production sites, markets, and even consumer environments. Lab-on-a-chip platforms, lateral flow immunoassays, CRISPR-based diagnostics (e.g., SHERLOCK, DETECTR), and smartphone-integrated sensors are transforming microbial surveillance by reducing dependence on central labs (Pardee et al., 2016; Kellner et al., 2019). Moreover, AI and machine learning (ML) are being increasingly applied to enhance detection sensitivity, automate data interpretation, and predict contamination patterns. For example, AI-enhanced image analysis of microbial cultures or lateral flow assays has improved the precision of visual readings. At the same time, ML algorithms have been integrated with sensor data to forecast spoilage or pathogen risk in perishable foods (Zhang et al., 2022). Despite their promise, these innovations require rigorous validation, user-friendly interfaces, and supportive regulatory frameworks to enable broad adoption. Challenges include data standardization, cybersecurity in cloud-connected devices, and ensuring ethical use of AI-driven decision-making tools in food governance.

12.0. CONCLUSION

Ensuring the microbiological safety of food is a critical priority in public health and global food systems. This chapter has outlined the diverse sources and pathways of microbial contamination, along with the wide range of detection methods currently available, from conventional culture-based techniques to cutting-edge molecular diagnostics and biosensor technologies. Each method offers unique advantages depending on the food matrix, target organism, required sensitivity, and operational context. The integration of high-throughput sequencing, CRISPR-based

diagnostics, and lab-on-chip systems is particularly promising for achieving rapid, accurate, and field-deployable detection.

Moreover, the chapter emphasized the importance of employing appropriate sampling strategies, method validation, and adherence to international standards to ensure the reliability and comparability of results. The growing application of microbial detection data in risk assessment and food safety management, primarily through tools like HACCP and QMRA, underscores the strategic role of detection in preventing foodborne outbreaks. As the complexity of food supply chains increases and climate change introduces new microbiological risks, the ability to detect contaminants swiftly and accurately will remain a cornerstone of effective food safety systems. Continued investment in innovation, capacity building, and cross-sector collaboration will be essential for advancing detection capabilities and protecting global public health.

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