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Nanobiotechnology-Based Diagnostics for Antibiotic-Resistant Foodborne Pathogen

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ABSTRACT

The rapid emergence of antibiotic resistance in microbial foodborne pathogens poses a growing threat to human health. Recent advances highlight progress in the detection of the most prevalent and clinically significant organisms, including

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members of the Enterobacteriaceae family, *Escherichia coli*, *Salmonella*, *Campylobacter jejuni*, *Shigella*, and *Vibrio cholerae*. Updated diagnostic approaches now incorporate nanobiotechnology and advanced data analysis tools, enhancing traditional techniques such as optical assays and electrochemical systems. In combination with modern immunoassays, these innovations allow for earlier identification of contamination and more effective prevention of food spoilage.

Keywords: Microchips. Salmonella, Escherichia coli, PCR, ELISA

INTRODUCTION

Fresh fruits and vegetables, including those grown under organic cultivation, constitute a vital component of a balanced diet, providing key macro- and micronutrients necessary for maintaining human health. However, these same food sources present significant risks for pathogenic contamination when inadequate hygiene practices are employed during preparation and consumption. Animal-derived products, including dairy products, poultry, seafood, and red meat (Le Loir et al., 2003), demonstrate particularly high susceptibility to microbial contamination. Likewise, processed products, including fast foods and ready-to-eat meals, have been identified as important vehicles for the transmission of foodborne pathogens (Rickman et al., 2007). As a result, foodborne infections remain a major contributor to the global burden of infectious diseases. The transmission dynamics of foodborne pathogens are influenced by multiple variables, including host susceptibility factors, food processing methodologies, storage conditions, supply chain logistics, and final distribution practices (Deisingh & Badrie, 2005)

FOOD-BORNE PATHOGEN

Various types of foodborne pathogens are recognized as major contributors to foodborne outbreaks.

Enterobacteriaceae

Gram-negative rod-shaped bacteria, measuring 0.3 to 1.5 micrometers in diameter, primarily participate in respiratory and fermentative processes. These microorganisms serve as crucial microbiological indicators for assessing food quality, as they form colonies within the intestines of animals and are associated with food. Aptamers, artificial antibodies, are early detection and easily cooperate with novel multiplexed biosensors (Mishra et al., 2024). They are applied successfully for

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methane, hydrogen, and volatile acids production (Rusanowska et al., 2025). According to Anita et al. (2024) the *Enterobacteriaceae* immune the most resistant pathogens to antibiotics, thus becomes hazardous.

Escherichia Coli

Common microorganisms present in feces often serve as indicators of fecal contamination, typically involving a gram-negative bacterium. This bacterium is frequently implicated in food poisoning and urinary tract infections. Preventive measures to mitigate such risks include ensuring proper food handling by food workers, promoting personal hygiene, adequately cooking food, and utilizing treated water sources. Even a minimal presence of pathogenic *Escherichia coli* can significantly endanger human health, particularly among children, the elderly, and individuals with compromised immunity. Such infections are associated with a wide spectrum of clinical outcomes, including gastrointestinal inflammation, abdominal discomfort, diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, reactive arthritis, and, in severe cases, mortality. To ensure timely identification, several diagnostic approaches have been developed. These include optical, microchip-based, and electrochemical methods, alongside molecular techniques such as loop-mediated isothermal amplification (LAMP) and rolling circle amplification (RCA) (Xue et al., 2025).

Salmonella

Morphologically, these bacteria are gram-negative rods measuring approximately 0.5 μm in width and 1–3 μm in length. They are also heat-labile, with elevated temperatures capable of effectively inactivating them. Occurring in meat and eggs with high resistance to most antibiotics, exact detection method is required. The PCR, ELISA, and biosensing are most accurate (Bakin et al., 2025; Quiñones et al., 2025; Muslin et al., 2025).

Campylobacter

Campylobacter jejuni represents the predominant bacterial pathogen associated with gastroenteritis globally, characterised by acute diarrhea andpancreatic illness accompanied by severe abdominal cramping. This zoonotic pathogen primarily colonises the intestinal tract of livestock, particularly poultry and cattle, serving as

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the principal reservoir for human infection. Additionally, *C. jejuni* contamination occurs through waterborne transmission routes, particularly in environments with inadequate sanitation infrastructure. They can be easily detected using Clustered Regularly Interspaced Short Palindromic Repeats. The spraying of food with blue or blackberry extracts is an efficient method of prevention, according to (Higuera-Ciapara, et al., 2024)

Shigella

Shigella is primarily transmitted through contaminated food and water, but person-to-person contact is the most common mode of transmission. Infection with Shigella can lead to gastrointestinal disturbances, such as dyzyntery, bloody diarrhea accompanied by abdominal pain and fever. The highly efficient aptamers allow for detection in food (Guo, X. 2024). While the majority of cases resolve without complications, approximately 0.1% of infections can result in fatal conditions.

Vibrio Cholerae

Vibrio cholerae is the causative agent of cholera, a waterborne infection that may also be transmitted through contaminated fruits and vegetables. The disease is characterized by profuse watery diarrhea, which can rapidly progress to severe dehydration and, if untreated, may become fatal (Andoh et al., 2009; Rajwar et al., 2016). In situ-synthesized gene chip is most accurate and recommended method of detection (Abdelshafy et al., 2025).

Additional significant foodborne bacterial pathogens encompass specific strains of *Staphylococcus aureus*, *Bacillus anthracis* (characterised by anthrax toxin production), *Clostridium perfringens*, *Clostridium botulinum* (producer of the potent neurotoxin botulinum), *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Coxiella burnetii* (Ronald & García, 2013). Contemporary diagnostic approaches have incorporated multiplex quantitative polymerase chain reaction (qPCR) methodologies utilising TaqMan probe-based detection systems for simultaneous identification of these pathogens, including *Y. enterocolitica*, S. *aureus*, *Shigella spp.*, *Salmonella spp.*, *Listeria. monocytogenes*, *E. coli* O157:H7, *C. jejuni*, and *B. cereus*, within a single analytical procedure (Hodzic, Glavinic, and Wademan 2023). These bacterial species constitute primary causative agents of foodborne disease outbreaks; with

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their associated toxins contributing to severe clinical manifestations and public health burden (Alocilja& Radke, 2003).

BIOMARKERS IN MICROORGANISMS

Microorganisms can be categorized into distinct groups based on their cellular structures and differences in composition. For instance, cell-wall bacteria are further classified into gram-positive and gram-negative types, depending on their ability to stain during specific laboratory tests (Palecek, 2002; Mills et al., 2019; Takahashi, 2018; Hyun et al., 2018; Mempin et al., 2013; Slavov et al., 2017).

Metabolic Products

The accumulation of these charged compounds leads to detectable alterations in electrical conductivity, which can be measured using electrochemical methods (Webster et al., 2015). Such ionic changes influence the physicochemical characteristics of the culture environment. In addition, metabolites such as amino acids, nucleotides, and organic acids function as important biomarkers, providing insight into microbial viability and metabolic activity within food systems (Liu et al., 2018).

DETECTION OF FOOD-BORNE PATHOGENs

Detecting foodborne pathogens requires not only the identification of the causative microorganisms but also an understanding of their biological characteristics, implementation of control strategies, and reduction of infection risks. Conventional diagnostic approaches—such as cell culture, colony enumeration, microscopic observation, polymerase chain reaction (PCR), and immunological assays—have traditionally been employed for pathogen detection (Sirigireddy & Ganta, 2005; Smith & Osborn, 2009; Md Alahi & Mukhopadhyay, 2017; Mullis et al., 1986; Malorny et al., 2003). Quantitative PCR platforms integrate fluorescent labelling molecules that permit continuous observation of DNA synthesis through luminescent signal measurement (Cady et al., 2005; Rodríguez-Lázaro et al., 2005). Multiplex approaches offer considerable analytical advantages by enabling concurrent identification of various bacterial taxa during individual experimental procedures, achieved through deployment of multiple primer pairs designed against unique genomic targets (Poirel et al., 2011; Raj et al., 2011).Traditional PCR approaches demonstrate inherent constraints regarding differentiation between

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living and deceased bacterial populations. Reverse transcriptase methodologies overcome these restrictions through RNA template targeting, consequently delivering improved detection capabilities for metabolically functioning, living bacterial organisms (Yaron & Matthews, 2002).

Immunology-Based Methods

Antibody-mediated detection strategies constitute highly efficient techniques for microbial identification, offering exceptional target specificity alongside broad analytical versatility. Principal methodologies encompass magnetic particle-based separation systems, enzyme-linked immunoassay platforms, electrochemical detection arrays, current-measuring immunological assays, and associated analytical procedures. Magnetic separation protocols utilize antibody-functionalized paramagnetic particles that demonstrate selective binding affinity toward target microorganisms, facilitating efficient isolation from heterogeneous food matrices. Integration possibilities exist with complementary analytical systems, including magnetic bead enumeration devices, electromagnetic field sensors, and light microscopy platforms to achieve enhanced detection limits (Besse et al., 2002; Ruan et al., 2002).Enzyme-linked immunoassay techniques exploit highly specific antigen-antibody recognition events and have gained widespread acceptance for their analytical sensitivity and methodological flexibility. These platforms enable concurrent pathogen screening for multiple species—including Salmonella and Escherichia coli—while generating diagnostic outcomes within approximately sixty minutes (Crowther, 2000).Electrochemical detection approaches facilitate rapid bacterial identification at the molecular scale within contaminated food specimens and require significantly reduced analysis times compared to conventional cultivation methods, establishing their utility for food safety monitoring. Amperometric electrochemical immunoassay systems provide exceptional analytical precision for both quantitative enumeration and qualitative identification purposes. This methodology, evolved from traditional enzyme immunoassay principles, has established consistent operational reliability. Investigative studies focusing on *Staphylococcus aureus* identification achieved effective microbial enumeration at bacterial densities as low as 10 colony-forming units per millilitre throughout genuine contamination scenarios and deliberate bacterial introduction trials within

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food and milk specimen types, confirming methodological precision and detection capability performance (Tania et al., 2012).

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Ribosomal DNA amplification followed by restriction enzyme digestion constitutes a molecular taxonomic approach utilizing enzymatic cleavage proteins to produce organism-specific banding profiles derived from enhanced 16S ribosomal gene sequences (Vaneechoutte et al., 1998; Woese and Fox, 1977; Rajwar et al., 2016; Kim et al., 2006).

Biosensor

These platforms operate through molecular-scale target recognition, facilitating microorganism and pathogenic agent identification within food matrices (Byrne et al., 2009; Turner, 2000). Given their rapid response characteristics and analytical precision, these detection systems experience expanding utilization across food manufacturing sectors for maintaining product quality assurance and consumer safety protocols.

Several types of biosensors have been developed, each exploiting different transduction mechanisms. Optical biosensors, often based on fiber-optic technology, enable rapid detection of pathogens such as *Escherichia coli*, *Salmonella*, *Listeria*, and *Staphylococcus* spp. (Leung et al., 2007; Arora et al., 2011; Sharma & Mutharasan, 2013). Thermal biosensors detect temperature changes associated with biochemical reactions, while ion-sensitive biosensors utilize ion-sensitive field effect transistors for high-resolution detection (Mohanty &Kougianos, 2006). Electrochemical biosensors, including potentiometric, amperometric, and impedimetric platforms, are widely used to monitor the electrochemical responses of biological interactions (Iqbal & Gupta, 2012). Potentiometric biosensors have successfully identified *E. coli* in food matrices such as sandwiches (Pohanka &Skládal, 2008), while amperometric biosensors are employed on a larger scale for quantitative detection. Impedimetric biosensors, regarded as among the most powerful, can detect nucleic acids and pathogen-specific antigens with high sensitivity (Davis et al., 2005). Piezoelectric biosensors, on the other hand, enable rapid identification of peptides and nucleic acids in contaminated foods. More recently, emerging platforms such as MXene-based biosensors, which operate through electric potential changes, have shown

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promise in detecting pollutants in plants and soil (Sengupta et al., 2025). Collectively, these diverse biosensor technologies hold considerable potential for advancing food quality assurance and safety monitoring.

FOOD SAFETY AND QUALITY

Consumption of contaminated food is a significant cause of foodborne illnesses, which often manifest acute symptoms such as diarrhea (sometimes bloody), nausea, vomiting, or fever. These symptoms are primarily associated with gastrointestinal tract disturbances and nutritional imbalances (Devi Nelluri & Thota, 2018). To prevent foodborne diseases, it is crucial to prioritize the consumption of high-quality, safe food, which is essential for maintaining a healthy lifestyle at all stages of life. Food safety and food quality are paramount considerations for the well-being of communities. The cost of ensuring food safety and quality is a worthwhile investment, as it directly impacts the health of individuals. Affordable, accessible, and economical methods should be adopted for detecting pathogens in food and ensuring its quality through the implementation of efficient quality management systems. To address the challenges posed by foodborne diseases and outbreaks, professionals, policymakers, industry partners, families, and individuals alike need to collaborate and contribute to a robust, healthy food system. In this context, the World Health Organization (WHO) has developed global risk communication strategies and training materials to support countries in enhancing food safety education and evaluation programs (Rajwar et al., 2016).

CONCLUSIONS

Foodborne bacterial pathogens demonstrate increasing acquisition of antimicrobial resistance, necessitating the development of precise and reliable detection methodologies. Novel diagnostic approaches include advanced microfluidic devices, specialised microchip-based platforms, and aptamer-based detection systems, which collectively offer enhanced specificity and sensitivity for pathogen identification. Furthermore, the integration of machine learning algorithms with nanotechnology platforms represents a significant advancement in both detection capabilities and preventive strategies for food contamination control, enabling more sophisticated monitoring of pathogenic organisms and food spoilage processes..

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