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Aptamers as next-generation tools for real-time food monitoring

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Abstract
Pathogenic bacteria and chemical contaminants in food pose serious threats to human health, necessitating rapid, sensitive, and cost-effective detection methods. Conventional approaches, including microbial culture, molecular assays, and immunoassays, are limited by long turnaround times, high costs, labor intensity, and reliance on skilled personnel. Aptamer-based biosensors (aptasensors) have emerged as promising alternatives due to their high specificity, stability, and scalable chemical synthesis. Aptamers, generated via SELEX (Systematic Evolution of Ligands by EX ponential enrichment), offer strong target affinity and versatility in detecting pathogens, toxins, antibiotics, and heavy metals. Electrochemical and optical aptasensors have demonstrated effective detection of *Salmonella typhimurium*, *E. coli*, Listeria monocytogenes, aflatoxins, penicillin, kanamycin, Pb^{2+}, and Cd^{2+} with low detection limits. However, challenges such as aptamer degradation, chemical modification requirements, and regulatory barriers limit commercial adoption. Aptasensors represent a versatile and promising approach for enhancing food safety monitoring.

Keywords: Listeria monocytogenes, Aptamers, aptasensors, *Salmonella typhimurium*

Introduction
Pathogenic bacteria, such as Salmonella, pathogenic E. coli, Staphylococcus aureus, and Vibrio parahaemolyticus, pose serious health risks through contaminated food. Their contamination is often multi-type, widespread, and occurs at low levels (Ma *et al*., 2021) [19], making rapid detection essential. Conventional detection methods—microbial culture, molecular biology, and immunoassays have limitations. Culture-based techniques, though the gold standard, are slow, requiring multiple steps that may take several days to a week for species confirmation (Yadav *et al*., 2020) [41]. Molecular tests may yield false positives (Majdinasab *et al*., 2018) [20], and immunoassays like ELISA are labor-intensive, time-consuming, and expensive due to antibody production (Yue *et al*., 2021; Sakamoto *et al*., 2018) [43, 28]. Lateral flow assays face issues of lower sensitivity, cross-reactivity, and storage stability (Dey *et al*., 2023) [7]. Overall, these approaches are limited by high costs, long turnaround, and dependence on skilled personnel (Quintela *et al*., 2022) [26]. To overcome this, low-cost, on-site detection platforms have been developed, with aptamers gaining prominence in food safety and other applications (Mustafa *et al*., 2017; Silva *et al*., 2020) [22]. Aptamers are short, single-stranded (ss) DNA or RNA molecules, or sometimes peptides, that recognize and attach to specific targets with strong affinity and high selectivity because of their distinctive three-dimensional conformations (Syed *et al*., 2010) [33]. These aptamers are typically generated through the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) technique, which was first introduced in 1990 (Wang *et al*., 2024) [35].
Aptamers were first identified in 1990, and since then numerous research groups have exploited their binding capabilities to develop a wide range of target-specific aptamers. The term “aptamers,” originating from the Greek word aptus meaning “to fit,” was introduced by Andy Ellington and Jack Szostak, who independently developed the same basic selection approach (Ellington and Szostak. 1990) [10]. Aptamers are often referred to as “chemical antibodies” because of their strong and specific binding characteristics (Diao *et al*., 2018) [8].

In the SELEX process, a large library containing 10^{14}-10^{16} random oligonucleotide sequences is used, from which candidate aptamers are selected based on their ability to bind a particular target molecule (Manea *et al*., 2024) [21].

SELEX: A Strategy for Aptamer Selection

SELEX stands for Systematic Evolution of Ligands by EXponential enrichment. It is a laboratory technique used to generate aptamers, which are short DNA or RNA sequences that can specifically bind to a target molecule (like proteins, small molecules, or even cells). There are many type of SELEX cycle (Stoltenburg *et al*., 2007) [32].

Cell-SELEX

Unlike other SELEX variants, the cell-SELEX approach isolates aptamers directly against intact cells, ensuring that the cell-surface molecular targets remain in their native conformations and natural functional states (Sefah *et al*., 2010) [29]. The procedure begins with the generation of a diverse single-stranded DNA library, typically comprising around 10^{13}-10^{15} distinct sequences flanked by primer-binding regions to facilitate PCR amplification (Chinchilla-Cárdenas *et al*., 2024) [6]. This library is then incubated with the target cells—such as live *L. pneumophila* serogroup 1 (SG1)—under physiological conditions to enable binding to unaltered surface epitopes (Xiong *et al*., 2022) [39]. After incubation, non-binding sequences are removed through washing, while the bound aptamers are recovered by elution methods such as heat treatment or chemical denaturation (Kumar *et al*., 2017) [15]. To increase selection specificity, a counter-selection step is incorporated using closely related organisms, including *L. pneumophila* SG3, *L. anisa*, *L. micdadei*, and *F. bozemanae*, which helps eliminate aptamers that bind non-specifically (Xiong *et al*., 2022) [39]. The recovered aptamers are then PCR-amplified to produce a fresh pool for subsequent selection rounds. This iterative cycle of binding, washing, elution, counter-selection, and amplification is repeated usually for 8-12 rounds with progressively stricter conditions to enrich highly specific, high-affinity aptamers (Chinchilla-Cárdenas *et al*., 2024) [6]. After sufficient enrichment, the final aptamer pool is cloned and sequenced, and individual aptamers are evaluated for their binding strength and specificity.

Magnetic-Bead SELEX

Magnetic SELEX is a modified SELEX technique in which the target molecule is immobilized on magnetic beads, and the magnetic property allows rapid separation of aptamer-target complexes from unbound sequences, making the selection process faster and more efficient (Manea *et al*., 2024) [21]. These Magnetic beads are composed of a core made from iron oxide nanoparticles, typically magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). These magnetic cores impart superparamagnetic properties to the beads, enabling them to be manipulated using an external magnetic field Lee *et al*., (2018) [16]. To prevent oxidation and provide a stable surface for functionalization, the magnetic cores are coated with silica or polymers, which also enhance the beads' biocompatibility and dispersibility in aqueous solutions. Mylkie *et al*., (2021) [23]. A variety of chemical functional groups, such as amines, carboxylic acids, epoxy, and aldehydes, can be introduced on the coating surface of magnetic beads to enhance stability, wetting properties, binding flexibility, and enable covalent immobilization of proteins, enzymes, RNA, or DNA biomolecules Frickel *et al*., (2010) [11].

Antibody vs Aptamer

Aptamers, which are synthesized chemically, enable large-scale, cost-effective, and highly consistent production with very limited batch-to-batch variability (Zhou *et al.,* 2012) [44]. This contrasts with monoclonal antibodies, whose manufacturing relies on expensive and time-consuming cell-culture systems that frequently introduce lot-specific inconsistencies an issue that makes aptamer-based sensors more appropriate for low-cost, disposable, point-of-care use in both food safety and healthcare settings (Sequeira-Antunes and Ferreira, 2023) [30]. Additionally, aptamers exhibit advantageous physical characteristics: their compact molecular size allows for higher surface-density immobilization within the biorecognition layer, effectively broadening the biosensor's dynamic range and enhancing target-binding efficiency relative to larger antibodies (Arshavsky-Graham *et al*., 2022) [3]. Aptamers also demonstrate notable resilience under non-physiological conditions and can be precisely engineered with functional groups such as thiol (-SH) or amine ($-NH_2$) tags to support customizable immobilization strategies, including Au-S bond formation on electrode surfaces (Guo *et al.,* 2025) [12]. Importantly, even when subjected to stressors like elevated temperatures or harsh environments, aptamers can undergo reversible denaturation and subsequently refold upon cooling, fully restoring their binding capability—unlike antibodies, which often incur irreversible structural damage and loss of function during such regeneration processes (Song *et al*., 2012) [31].

Aptasensor Applications in Modern Food Safety Monitoring

Aptasensors employ a variety of analytical approaches to convert specific biomolecular interactions into detectable signals. These systems are generally classified according to the underlying physical transduction mechanism, with the most common being optical, electrochemical, and mass-based methods. Among the technologies progressing toward commercial use, electrochemical, optical, mass-sensitive, and micromechanical aptasensor platforms remain the most prominent (Léguillier *et al*., 2024) [17].

Electrochemical Aptasensors: Precision and Miniaturization

Electrochemical detection is widely used in biosensing due to its simple fabrication, miniaturization potential, and compatibility with lab-on-chip systems (Léguillier *et al*., 2024) [17]. Electrochemical aptasensors commonly rely on cyclic voltammetry, differential pulse voltammetry, and electrochemical impedance spectroscopy (Léguillier *et al*., 2024) [17], and they often achieve higher sensitivity and lower detection limits than other sensing methods (Phopin *et al*., 2020) [24].

Their superior performance largely stems from nanomaterial integration. Nanostructures such as AuNPs, rGO, and carbon composites enhance electron transfer, improve electrocatalysis, and offer large surfaces for aptamer immobilization (Léguillier *et al*., 2024) [17]. These materials amplify signals and further lower detection limits by boosting surface area and catalytic activity (Yousef *et al*., 2022) [42]. Engineered nanomaterials, including N-doped

porous carbon and gold nanostars, also increase stability and target enrichment, enabling reliable detection even in complex real-world samples (Wang *et al*., 2022) [35]. For example Study by (Ahmadi *et al*., 2022) [1] used an rGO-AuNP-modified pencil graphite electrode and was characterized using SEM, FTIR, CV, and EIS techniques. It achieved a wide linear detection range of 0.5-800 ng/L and a very low detection limit of 0.3 ng/L for aflatoxin M1, with performance successfully validated against HPLC.

Optical Aptasensors: Readout Versatility and Label-Free Options

Optical aptasensors detect target binding by monitoring changes in light properties and use formats such as fluorescence, chemiluminescence, colorimetry, SPR, LSPR, and SERS. Colorimetric assays are especially suitable for Point-of-Care testing because they are simple and allow visual interpretation (Thanunchai *et al*., 2025). AuNP-based colorimetric aptasensors can rapidly and selectively detect whole bacteria like E. coli O157:H7 and Salmonella typhimurium in under 20 minutes without complex pretreatment (Wu *et al*., 2012) [38].

There are many example of optical aptasensors like a fast and simple structure-switching fluorescent aptasensor was developed for the quantitative detection of Aflatoxin M1 (AFM1) in milk, showing a linear range of 1-100 ng/mL and a detection limit of 0.5 ng/mL, with excellent recoveries of 93-101% in spiked samples Qiao *et al*., (2021) [25] and simple and high-performance FAM-labeled aptamer fluorescent sensor was developed for rapid on-site detection of cadmium (Cd^{2+}), showing a linear range of 5-4000 ng/mL and a detection limit of 1.92 ng/mL, which is below the WHO and EPA limits for drinking water Liu *et al*., (2021). Label-free techniques such as SPR and LSPR further simplify detection by eliminating the need for fluorescent or enzymatic labels, making them well-suited for complex biological samples (Wu *et al*., 2012) [38].

Studies on the Detection of Foodborne Pathogens

A variety of aptasensor platforms have shown strong potential for foodborne pathogen detection. A visual aptasensor enabled on-site identification of *S. typhimurium* in milk, demonstrating a linear response from 10^2 to 10^6 CFU/mL and high selectivity, with sensitivities of 2.4×10^2 CFU/mL in PBS and 2.8×10^3 CFU/mL in actual milk samples.

Another fluorescence-based approach was developed for detecting *Escherichia coli* in raw beef using Fe_3O_4@Au nanoparticles combined with borophene quantum dots, achieving detection limits of 3 CFU/mL in PBS and 20 CFU/mL in spiked meat samples (Alzahrani, 2025).

Additionally, a novel colorimetric aptasensor employing Fe_3O_4@MIL-100(Fe) magnetic nanoparticles functionalized with a specific aptamer exploited their intrinsic oxidase-like activity for the detection of *Listeria monocytogenes*. This system provided a broad detection range of 10^2-10^7 CFU/mL and a remarkably low limit of detection of 14 CFU/mL (Du *et al*., 2024) [9].

Studies on the Detection of Toxins and Antibiotic Contaminants

Several aptasensor platforms have been developed for detecting chemical contaminants in milk. An electrochemical aptasensor using a PEN-specific DNA aptamer (P-11-1) combined with AuNP-Fe_3O_4 nanocomposites enabled simultaneous detection of penicillin antibiotics, achieving a low detection limit of 0.667 nM and a broad linear range from 2 to 10,000 nM (Hu *et al*., 2024) [13].

Another electrochemical system incorporating reduced graphene oxide and gold nanoparticles was designed for monitoring Aflatoxin M1, providing a highly sensitive detection limit of 0.3 ng/L (Ahmadi *et al*., 2022) [1].

Additionally, a fluorescent aptasensor based on a split aptamer and DNA-templated silver nanoclusters, combined with Exonuclease I amplification, enabled highly sensitive detection of kanamycin in milk, reaching an ultra-low detection limit of 1.07 nM (Xu *et al*., 2025) [40].

Studies on Aptasensor-Based Detection of Heavy Metals

A highly sensitive, enzyme-free fluorescent aptasensor was developed for detecting the toxic lead ion (Pb^{2+}) in fish, shrimp, and milk. The system employed a ZIF-8@AuNPs metal-organic framework/gold nanoparticle hybrid as an efficient fluorescence quencher, achieving an exceptionally low detection limit of 0.24 pM (Rouhi *et al*., 2024) [27].

Critical Gaps

Native aptamers—especially RNA-based ones—are highly prone to nuclease degradation, making them unstable in real samples (Kratschmer *et al*., 2017) [14]. This issue becomes more severe in food safety testing because food matrices such as milk, meat, and produce extracts naturally contain nucleases that can rapidly break down aptamer strands (Zhou *et al*., 2012) [44].

To improve stability, aptamers often require extensive chemical or structural modifications after SELEX, transforming them from simple oligonucleotides into engineered molecules and increasing production complexity and cost (Guo *et al*., 2025) [12].

Another major barrier to commercial use is the absence of clear regulatory approval pathways. Although many aptamer-based sensors show strong performance in research settings, they still struggle to gain acceptance for routine food safety testing, slowing their industrial adoption (Boro *et al*., 2025) [4].

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