

Updated Review

Applications of Aptamers in Food Safety and Quality Control: A Comprehensive Review.

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1. Background

Food safety has long been a serious global issue that not only imposes a huge burden on the economy, but also greatly threatens people's lives and health. According to the World Health Organization (WHO), contaminated foods cost economically lost about \$110 billion per year in production damage and medical treatments.

Moreover, nearly one in ten people in the world (ca. 600 million) get sick from taking food contaminated with bacteria, viruses, parasites, & chemicals substance, resulting in 420,000 deaths each year. More than 200 diseases are caused by ingesting these contaminated foods (1). In Bangladesh, food safety issues are particularly severe, stemming from inadequate sanitation, poor hygiene practices, extensive food adulteration, and limited monitoring resources. Around 30 million Bangladeshis suffer from foodborne illnesses per annum, predominantly acute gastroenteritis, highlighting the burning need for rapid detection and mitigation strategies to associate with global priorities in reducing food and nutritional hazards, safeguarding public health, and minimizing economic and social impacts (2). Food safety is critically important for human health and social stability. Each incident of food adulteration or contamination causes severe physical harm to affected individuals and exerts widespread societal impacts (3). Contamination of food may arise in any of the production, processing, storage, transportation, and consumption, while closely related to water, air, soil and other environments. Therefore, controlling food quality is essential to regulate food borne diseases and to satisfy the requirements of human health (1).

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Currently, high-performance liquid chromatography (HPLC), mass spectrometry (MS), and liquid chromatography-mass spectrometry (LC-MS) are considered the most reliable for analyzing small molecule residues in food safety and typically yield highly accurate results (3). However,

traditional detection approaches may be hindered by complexities, time-intensive protocols, requirement for specialized instrumentation & expertise, potentially limiting its practical applicability (4). Thus, establishing rapid, accurate & truly deployable on-site food safety detection methods is essential for effectively controlling adulteration and contamination at the source (3). Given Bangladesh's significant food safety challenges, adopting rapid and effective detection methods is particularly essential (2).

Aptamers, essentially nucleic acid molecules obtained through 'Systematic Evolution of Ligands by Exponential Enrichment (SELEX)' technology, have garnered widespread attention since their discovery in the 1990s due to their exceptional performance. Serving as specialized molecular tools, aptamers possess the ability to selectively interact with specific target molecules, forming stable complexes with high specificity and affinity, thereby demonstrating multifunctionality. Compared to traditional antibodies, aptamers offer advantages such as small molecular size, ease of modification, cost-effectiveness, and high stability. With a broad recognition spectrum, aptamers exhibit the capability to target various entities, encompassing ions, small molecules, proteins, and cells (4). Aptamers are artificial antibodies and single-stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA) (5). In the binding system, the aptamer undergoes adaptive conformational changes according to the structure of the target, forming special structures such as hairpin, pseudoknot, bulge, G-quadruplex, and so on. Stable complex is formed with target through hydrogen bonding, van der Waals force, base stacking force, electrostatic interaction, or hydrophobic interactions (6).

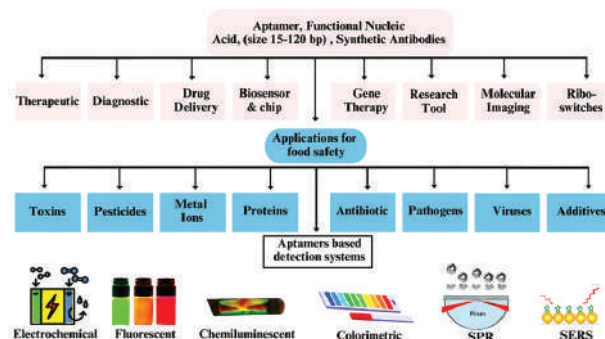


Fig.1. Application of aptamers in food safety detection systems (7)

In this review, we aim to overview the importance the application of aptamer in food safety and quality control, particularly in Bangladesh. Despite the global advancement in aptasensors technology, its application in the food safety framework of developing nations like Bangladesh remains significantly unexplored. There is a significant need to bridge the gap between sophisticated laboratory research and on-site detection of local food contaminants.

2. Antibody vs Aptamer

Aptamers, which are synthesized chemically, enable largescale, cost-effective, and highly consistent production with very limited batch-to-batch variability (8). However, the generation of antibodies is time-consuming, and the storage condition is relatively strict. Aptamers are single-stranded (ss) DNA or RNA molecules that can mold into specific structures and bind to their targets with high affinity and specificity, which can be regarded as alternative molecule recognition elements to antibodies. They have demonstrated lots of advantages compared to antibodies, such as wide range of targets, good stability, little batch-to-batch differences and low cost (9). Moreover, aptamers exhibit advantageous physical characteristics: their compact molecular size allows for higher surface-density immobilization within the biorecognition layer, successfully broadening the biosensor's dynamic range and enhancing target-binding efficiency relative to larger antibodies. A notable resilience under non-physiological conditions are also demonstrated by Aptamers and can be precisely engineered with functional groups such as thiol (-SH) or amine (-NH₂) tags to support customizable immobilization strategies, including Au-S bond formation on electrode surfaces (8). The nonstop progress of aptasensors furnishes robust instrumentation and methodological support for molecular recognition, biosensing applications, pharmaceutical investigations, targeted therapeutic interventions, and other fields. (4)

3. Role of Aptamer in detecting food contaminants:

i) Detection of foodborne pathogens or microorganisms:

Microbial hazards present a substantial threat to public health and food safety in Bangladesh as it is a country renowned for its rich culinary traditions. It faces challenges from a range of pathogens, including bacteria, viruses, fungi, and parasites, that frequently contaminate the supply chain at multiple 'Critical Control Points' (2). Because food-borne pathogens risk human health via polluted food there is an urgent demand to establish convenient, sensitive, cost-effective, and proper detection techniques to monitor pollution in food (10).

The usual method of microbiological detection is to prepare culture medium for culturing micro-organisms, then the suspected pathogenic bacteria are isolated and get biochemical identification. Promoting bacteria step by step

is the method of detecting Salmonella in food which generally can be divided into four procedures (11).

The availability of aptamers with good stability and cost efficiency to specifically identify different pathogens opens the door to detection methods that satisfy the aforementioned requirements, mainly through their integration into bioassays or biosensors (10).

In addition to common optical and electrochemical aptamer biosensors, aptamer can be used to detect microorganisms in combination with many other techniques such as the surface enhanced Raman scattering and fluorescence resonance energy transfer. The development and utilization of these technologies make the application of aptamer not limited to optics or electrochemistry. In the field of detection, aptamer technology has a very wide range of applications (11).

Detection of foodborne microorganisms with aptamer can be done through two approaches. Aptamers can be selected against the whole cell or against a part of cell surface e.g., Outer membrane proteins. Several aptamers have been selected mostly against the common foodborne bacteria, namely Salmonella, E. coli, Listeria monocytogenes, Vibrio Parahaemolyticus, Staphylococcus aureus, Shigella sonnei and food borne virus Norovirus. Aptamers selected against pathogenic bacteria are incorporated into some of the innovative assays and biosensor platforms that can effectively detect the bacteria in food matrices. Whole SELEX cell was applied against live E. coli O157:H7 pathogenic strain that was captured by an anti-E. coli antibody conjugated on magnetic bead (12).

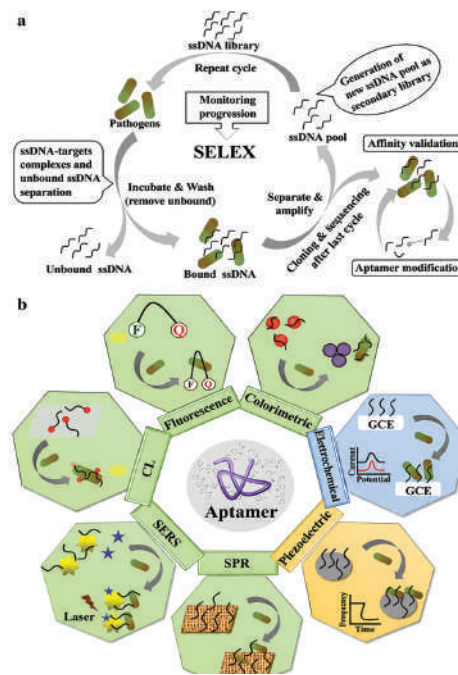


Figure 2: a) Fundamental process of aptamer selection, b) Schematic illustrations of apter-assays and aptasensors for pathogenic bacteria detection (10)

Table 1: Example of aptasensors & assays against different types of food contaminants (12)

Type	Analyte	Food sample	Aptamer type	Aptasensors/ Assay	LOD
Compounds generated	Histamine	–	DNA	Apta-PCR Assay	18 pM
During processing	Acrylamide	–	DNA	AuNPs-based colorimetric aptasensor	0.390 μ M
Leaching from Packaging material	Bisphenol A	Water	DNA	Nonconjugated gold nanoparticles and CdTe quantum dots-based fluorescent Assay	1.86 ng/mL
				Cationic polymer-aggregated gold nanoparticles	1.50 nM
				Based colorimetric aptasensor	
				Aptamer/Graphene Oxide FRET Biosensor	0.05 ng/mL
	Melamine	Water	DNA	Fluorescence Apta-assay	6.32 nM
Adulterant	Melamine	80% whole	DNA	Structure-switching aptamer-based Sensor	0.53 μ M
		milk			(67.5ppb)
	Melamine	Whole Milk	DNA	Fluorescence Aptasensors	0.12 μ M
	Melamine	Milk	DNA	aptamer-modified SERS Nano sensor	1.0 pg/mL.
	Urea	Milk	DNA	AuNP-based fluorescence aptasensors	20 mM
Preservative	Malachite green	Fish	levo (L)-	Fluorescence Assay	47.7 nM
			RNA		
			DNA	Colorimetric Assay	16.7 μ g/kg
			RNA	Colorimetric Aptasensors	15.95 nM
	Salicylic acid	Plant extracts	DNA	Structure-switching aptamer-based Nano sensor	0.1 μ M

ii) Detection of Mycotoxins:

Mycotoxins inescapable contaminants in environment, food and animal-feed, pose significant threats to human and animal health (4). Mycotoxins are ubiquitous that easily enters processed foods. cereals, beans, fruits, nuts, corn, rice, vegetable oils and several other food stuff that commonly contaminated with mycotoxins. Therefore, its accurate detection remains critical for food safety issues (1). Reportedly, in Bangladesh, mycotoxins signify a persistent food safety challenge, primarily produced by molds like *Aspergillus*, *Penicillium*, and *Fusarium* species. Mold growth and mycotoxin production in agricultural commodities, including nuts, grains, oil-seeds, and animal products, viz., milk and cheese- which Bangladeshi people preferred much more plausibly because of warm and humid conditions (2).

Mycotoxins- a group of common toxic contaminants in food- remain extremely immunosuppressive, terato-genic and

carcinogenic, even in trace amount. Due to high chemical and thermal stability, mycotoxins can continuously gather and exist in organisms via the food chain, posing a huge threat to human health and safety. Aflatoxin B1 (AFB1) is the most toxic and dominant among them, which is designated as group 1A carcinogen, while ochratoxin A (OTA) designated as group 2B carcinogen. So, it bears major significance and necessity to exploit efficient biosensors for on-site mycotoxin detection in large amount of food samples (9). AFB1 produced by fungus *Aspergillus* remains one of the most harmful toxins labelled as group I carcinogen for humans as designated by the International Agency for Research on Cancer (IARC). In the European Union the maximum permissible limit of AFB1 set for foodstuff is 2 ppb (2 μ g/kg). Notably, many aptamer-based sensors and assays are now been available to detect AFB1 with the limit of detection it in much lower than the EU-specified limit (12).

iii) Detection of Heavy Metals:

In Bangladesh, chemical food safety hazards present a serious challenge particularly in terms of food supply that dangerously affect country's public health. Further, with a growing population dependent largely on agriculture, the concern like as pesticide residues and, thus, contamination with heavy metal and over-use of unregulated chemicals, per se. Misuse of food additives/preservatives, in addition, often contributes to long-term public health risks for such consumers (2).

Apart from these, industrialization, urbanization, growing population and aging infrastructure in Bangladesh along with intensification of agricultural activities have increased heavy metals entering water threatens its soil causing serious environmental pollution. Moreover, heavy metal pollutants, like arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), silver (Ag), copper (Cu), etc. have been evoked as growing global concern because of being non-biodegradables which are absorbed by biological organisms. These metal ions pose a negative effect on the aquatic ecosystem and humans, leading to the oxidative deterioration of biological molecules, cell malfunction, and disturbance of the metabolism. Long-term exposure to heavy metals in small concentrations is reported to cause various diseases, i.e., cancer, neurological disorders, and nutritional immunity/bacterial infection. The real-time detection of heavy metal ions is important for biological and environmental monitoring (13). Heavy metals pose a significant health risk in Bangladesh due to the widespread contamination of commonly consumed foods from unregulated industrial activities. Industrial wastewater irrigation contaminates vegetables with toxic metals such as Pb, Cd, Cr, Ni, and As, exceeding the MRLs and posing carcinogenic, mutagenic, and teratogenic health threats (2).

4. Future Perspectives and Prospects

Towards a productive aim of strategic integration of Aptamer technology using advanced sensing modalities specifically, Surface-Enhanced Raman Spectroscopy (SERS) remains most appropriate for molecular fingerprinting, colorimetric assays for rapid visual screening, electrochemical sensors for precise quantification, and Fluorescence based platforms for high-sensitivity detection. Thus, aptamer research which definitely to be formally initiated in Bangladesh, with growing institutional interest to be explored in this field, which will provide a transformative framework for food security in Bangladesh.

Such potential for aptamer technology, underscored by successful studies in China, demonstrating its efficacy in multi-target detection (multi-modal approach) will not only ensure ultra-sensitive detection of trace-level contaminants but also offers the versatility desirable for both high-end lab analysis and affordable on-site field testing. However,

practical deployment of these sensors may have few limitations like: sensitivity to country's tropical environmental conditions, potential signal interference from complex food matrices and high initial costs of research and development.

Moreover, along with formation of specific 3D tertiary structure, electrostatic attraction arises due to polyanionic nature of aptamer helps in binding to positively charged heavy metal ions, although simple structure and single binding site of heavy metal possess disadvantages in aptamer selection. Aptamer specific to Hg^{2+} are generally T-rich that selectively binds to it through formation of a stable T- Hg^{2+} -T base pair. Such Hg^{2+} Specific aptamer has been utilized in development of a dual-channel biosensor that can simultaneously generate SERS and fluorescence signals for Hg^{2+} detection in spiked tap water and milk samples (12).

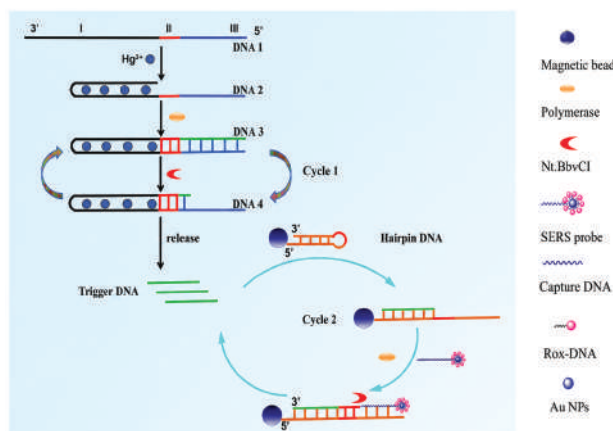


Fig. 3 Schematic representation of the SERS aptamer sensors designed for detection of Hg^{2+} (14).

Finally, by synergizing these cutting-edge techniques, Bangladeshi food scientists can establish a resilient, transparent and globally competitive food safety system that effectively safeguards public health. Therefore, continued development of aptamers-SERS will make trace tracking of more metal ions possible (viz., Pb^{2+} - which is one of most common heavy metals in food being carcinogenic and teratogenic (rapid and sensitive Pb^{2+} detection method by modulating the reduction of AuNPs) (1).

5. Conclusion:

Food safety is a major public health and social problem in many countries of the world including Bangladesh (BD) due to high-risk foodborne pathogens, heavy metals and mycotoxins being major threats to country's safety. Likewise, preservation of food safety in BD remain an enduring concern mediated by complex interplay of socio-economic status and environmental factors. Key

obstructions to such situation include deficient public literacy particularly on hygiene, deliberate chemical adulteration and pervasive misuse of agrochemicals, consisting of anti-biotics and pesticides.

All these aforementioned issues are intensified by country's tropical climate that often facilitates rapid microbial proliferation in BD, leaving out institutional weaknesses. These remain specifically fragmented regulatory oversight owing to multiple regulators involvement, inconsistent rules (making compliance complex). Overlapping authority (often contradict with each other), Compliance burden (higher costs only due to duplicate reporting and conflicting obligations), staying away from Risk of gaps (crucial issues fall through cracks of clear responsibility). All these include traceability and administrative inefficiencies that often obstruct comprehensive food safety management.

Addressing these systemic vulnerabilities requires a multifaceted national strategy focused on enhancing public awareness and strengthening the regulatory and infrastructural landscape to mitigate widespread contamination and misbranding risks. Aptamer is one of them to prevent this problem.

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