An Update on Aptamer-Based Multiplex System Approaches for the Detection of Common Foodborne Pathogens

Omar Mukama[1,2] · Jean Paul Sinumvayo[1] · Muhammad Shamoon[3] ·
Muhammad Shoaib[3] · Henriette Mushimiyimana[1] · Waseem Safdar[3] · Leo Bemena[1] ·
Peter Rwibasira[2] · Samson Mugisha[1] · Zhouping Wang[3]

Abstract Foodborne ailments constitute a public health challenge and pose an incredible economic burden in healthcare system around the globe. This dilemma has urged authorities and other entities working in field of food quality control and supply chain to play a pivotal role in ensuring food safety. Analytical strategies have been developed using numerous systematic evolution of ligands by exponential enrichment (SELEX) methods to assure food safety. High-affinity and high-sensitivity ssDNA and RNA aptamers against pathogens have emerged as a novel strategy, as compared to the more resource-demanding and complicated biochemical test-based approaches. Thus, this review aims to focus on some methods used in the selection of specific bare, modified, and conjugated aptamers and on the further analysis of selected aptamers using flow cytometer or post-SELEX modifications for enhanced detection of frequently diagnosed foodborne bacteria such as *Bacillus* sp., *Campylobacter jejuni*, *Escherichia* sp., *Salmonella* sp., *Staphylococcus aureus*, *Shigella* sp., *Listeria monocytogenes*, and *Streptococcus pyogenes* and/or targeting their cell components towards attaining fast, sensitive, and selective methods for the detection of pathogens in food(s) or other sources.

Abbreviations

FAM	6-Carboxyfluorescein
ATCC	American Type Culture Collection
FluMag-SELEX	Aptamer fluorescent labeling and magnetic beads immobilization
AM-ECL	Aptamer-magnetic bead-electrochemiluminescence
CE	Capillary electrophoresis–SELEX
CDC	Centers for Disease Control and Prevention
dPa	Deoxyribonucleoside triphosphate 4-propynylpyrrole-2-carbaldehyde
Px	Diol-modified 2-nitro-4-propynylpyrrole
DCE	DNA capture element
ELASA	Enzyme-linked aptamer sedimentation assay
ECDC	European Centre for Disease Prevention and Control
FITC	Fluorescein isothiocyannate
FRET	Fluorescence resonance energy transfer
FACS	Fluorescence-activated cell sorting
HTS	High-throughpuT-SELEX
Ds	Hydrophobic base 7-(2-thienyl) imidazo[4,5-b]pyridine
MREs	Molecular recognition elements
NECEEM	Non-equilibrium capillary electrophoresis of equilibrium mixtures
OMPs	Outer membrane proteins
QD	Quantum dot

✉ Omar Mukama
momary05@yahoo.fr

✉ Zhouping Wang
wangzp@jiangnan.edu.cn

[1] Key Laboratory of Carbohydrate Chemistry and Biotechnology, School of Biotechnology, Jiangnan University, Wuxi 214122, People's Republic of China

[2] Department of Applied Biology, College of Science and Technology University of Rwanda, Avenue de l'armée, P.O. Box: 3900, Kigali, Rwanda

[3] State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi 214122, People's Republic of China

SELEXp	Systematic evolution of ligands by exponential enrichment
SEA	*Staphylococcus aureus* enterotoxin A
SEB	*Staphylococcus aureus* enterotoxin B
SERS	Surface-enhanced Raman spectroscopy
TECS-SELEX	Target expressed on cell surface-SELEX
UCNPs	Up conversion nanoparticles
ROX	X-rhodamine

Introduction

Foodborne pathogens are life threating to a community in general and particularly to people susceptible to disease. Common causative agents include bacteria, viruses, parasites, and other microbes. Pathogenic microorganisms easily find their way into a food and ultimately into consumer, especially in an unhygienic environment in a food chain. Poor conditions in a food production chain are a major source of unsafe food, often with a considerable load of pathogens which could cause severe illness. Recent US Centers for Disease Control and Prevention reports show that 2.2 million people die every year, of which 1.9 million are children due to pathogenic bacteria: specifically, *Bacillus* sp. (*cereus, thuringiensis*, and *anthracis*), *Campylobacter* sp., *Escherichia* sp., *Listeria* sp., *Salmonella* sp., and *Staphylococcus* sp. are associated with foodborne and waterborne disease outbreaks (Control CfD 2013; Sett 2012). Therefore, to ensure food safety for consumers, a strict and reliable quality control and assurance approach must be implemented. To combat the ever-increasing succession of unsafe food norms, several analysis-based food safety measures have been developed such as immunological methods (conventional ELISA), immunomagnetic electrochemiluminescence assay, time-resolved fluorescence assay, rolling circle amplification, and strand displacement amplification. Despite all these innovations, many challenges exist in developing sensitive and reliable analytical methods with fast detection of specific food hazard(s) (Amaya-Gonzalez et al. 2013; Hayat et al. 2013; Litao Yang 2007; Velusamy et al. 2010). The ineffectiveness of non-nucleic acid based techniques is associated with complicated handling procedures, strict laboratory protocols, high cost, and significant susceptibility to change in conditions (Tombelli et al. 2007). Aptamers and their biosensor-derived techniques rapidly detect food contaminants at extremely low levels yielding a promising pathogen detection approach (McKeague and Derosa 2012; McKeague et al. 2014; Xu et al. 2011). Aptamer-based biosensors play a pivotal role to lessen this apprehension, since they are ideal candidates to improve testing. Use of aptamers in foodborne pathogen detection was first reported in 1990 (Tuerk 1990a), since then numerous biosensors have been designed for different targets from agri-food, environmental, pharmaceutical, and therapeutical origin (Beier et al. 2014a; Scognamiglio et al. 2014; Thiel and Giangrande 2009). However, still few putative techniques in detection of parasites (*Giardia lamblia*, *Taenia saginata*, *Taenia solium*, *Entamoeba histolytica*, *Cyclospora Cayetanensis*, *Pseudo terranova* spp., *Sarcocystissui hominis*), fungi (yeasts, molds), and viruses (Hepatitis A, Avian influenza, Rotovirus) and some bacteria (only M-type 11 *Streptococcus pyogenes*) are implemented.

In vitro selection and production attempts of bare and conjugated aptamer biosensors range from microbial detection to metabolic pathway study towards therapeutic usage (Bini et al. 2011). They are developed using systematic evolution of ligands by exponential enrichment (SELEX) protocol and bind to the target by inducing the duplexes dissociation and single strand DNA/RNA folding in a unique 3-D structure. This is achieved in two different ways namely specific ligands and/or through Watson–Crick complementary strand binding (Marty and Hayat 2014). They possess high affinity and specificity binding to numerous target molecules namely inorganic ions (Zn^{2+}), small molecules (biotin),organic dyes (malachite green), nucleotides (ATP), amino acids (citrulline, arginine), neutral disaccharides aminoglycoside antibiotics, proteins, large glycoproteins (CD4), viruses, anthrax spores (Zhijun Guoa 2011), cells (Drabovich 2009), toxins, drugs, low-molecular-weight ligands (Subramanian Viswanathan 2008), environmental pollutants, and disease biomarkers (Mei 2013).

Cell-SELEX provides flexibility in the detection of various cell types (bacteria, viruses, cancer cells) and is now used in the identification of the unknown cells' surface biomarkers (Kim et al. 2013c). Recently, modified SELEX protocols based on traditional SELEX method have significantly improved to advance the application of aptamer technology in various fields (Xu et al. 2011). SELEX process is modified to obtain aptamers with specific features towards definite uses (Stoltenburg et al. 2007). Chemical modifications of aptamers depend on their applications, primarily to increase their stability against biological fluids, introducing reporter molecules and/or functional groups and immobilization (Stoltenburg et al. 2005b). The immobilization of modified aptamers on the surface of biosensors through various fixation methods such as direct attachment (a biosensor's gold electrodes bind to thiol-modified aptamers), covalent binding (amino group-modified aptamers react covalently with different chemical groups (amino, carboxyl, hydroxyl, etc.), and biocoatings (avidin-coated biosensor bind with high specificity and affinity to biotin-labeled aptamers) exert impacts on the properties of biosensors which, in turn, enhance the performance of aptasensors (Zhou et al. 2011). These oligonucleotides are able to form a specific hairpin loop allowing them to recognize a variety of targets even for closely related pathogenic bacteria (Table 1). Though all improvements, aptamer technology still needs refinements to obtain aptamers with target specificity and strong binding properties in different environmental

Table 1 Recent report(s) on emerging foodborne pathogens detection by aptamers

Causative agents	Aptamer[a]	Sample(s)	Sensitivity[b]	Targeting part	Ref.
Bacteria					
Escherichia coli O157:H7	ssDNA/FITC-ssDNA-PDA[c]	Feces	10^4~10^8	LPS binding	So et al. 2008; Wenhe Wu and Wei Ye 2012
Staphylococcus aureus	FAM-ssDNA	Food, water	10^4~10^6	Cell surface-associated proteins, teichoic acid recognition	Abbaspour et al. 2015; Baumstummler et al. 2014; Chang et al. 2013a; Moon et al. 2015
Yersinia pestis	ssDNA	–	–	LOS and F1 antigen targeting	Fernandez 2006; Radi 2011
Vibrio cholera	ssDNA	Pure culture	4.2×10^2	Cell surface antigens binding	Zhang et al. 2015
Shigella sonnei	ssDNA	Strain	10^9	IpaH protein	Elham Masoudipour et al. 2011
Shigella dysenteriae	FAM-ssDNA	Strain	10^2~10^7 ($Kd = 23.47 \pm 2.48$ nM)	Whole cell	Duan et al. 2013b
Salmonella enteritidis	Polyvalent ssDNA	Strain	10^8	Aptamer–cell epitope interactions	Park et al. 2014a; Wei Wu 2015
Salmonella typhimurium	-ssDNA polymer Au@AgNPs-apt 1-target-apt 2-ROX complex	Strains	~10–10^6 –15	Whole cell	Duan et al. 2016; Yuan et al. 2014
Listeria monocytogenes	ssDNA/ssDNA-digoxigenin	ATCC pure culture	10^2~10^7 ($Kd = 48.74 \pm 3.11$ nM)	Lipopolysaccharide, lipoprotein or outer membrane proteins	Duan et al. 2013a; Liu et al. 2014; Suh and Jaykus 2013
Pseudomonas aeroginosa	FITC/QD-ssDNA	Water	1–10^3	Whole-cell recognition	Kim et al. 2013a; Wang et al. 2011; Wang et al. 2012
Campylobacter jejuni	FAM-ssDNA ssDNA-Biotin-qPCR	Strains	$Kd = 292.8 \pm 53.1$ nM 1.1 $\log_{10}$/300 μL	Whole-cell detection System	Dwivedi et al. 2010a; Suh et al. 2014b
Streptococcus pyogenes	FAM-ssDNA	Strains	$Kd = 7 \pm 1$ nM	M-type surface proteins	Hamula et al. 2016
Parasites					
Cryptosporidium parvum	ssDNA	Spiked fresh fruits	150–800 oocysts	Oocyst detection	Iqbal et al. 2015
Anisakis spp.	ssDNA	Fish, fish-derived products	–	–	Baird et al. 2014; Mossali et al. 2010
Pseudoterranova spp.	ssDNA	Fish, fish-derived products	–	Oocysts	Mossali et al. 2010
Viruses					
Hepatitis C (HCV)	ssRNA, ssDNA		$Kd=142$–224 nM	Viral nonstructural proteins (core antigen) HCV NS5B protein	Kotaro Fukuda 2003; Lee et al. 2015
Hepatitis B (HBC)	ssRNA	–	–	P proteins(capsid)	Feng et al. 2011
Norovirus	ssDNA	–	Low picomolar range	Synthetic capsids and capsid protein VP1	Beier et al. 2014b; Giamberardino et al. 2013
Enterovirus	ssDNA	Lettuce	3.2–4.4	Virus-like particles (GI and gGII)	Escudero-Abarca et al. 2014

[a] Type + label

[b] cfu/mL

[c] Unpolymerized vesicles are made from polydiacetylene (PDA)

conditions like pH variation, nuclease presence, salt concentrations, and temperature (Tombelli et al. 2007; Tibor Hianik 2007). Hence, this review aims to cover comprehensively current implemented bare and conjugated aptamer-based multiplex techniques in detection of food pathogens. The putative aptamers will be discussed in the context of their selection through modified whole-cell SELEX, proceeding to the mode of action and culminating with their advantages towards more accurate and reliable bioanalysis.

## General Principle and Modified SELEX for Foodborne Pathogens

Aptamers against specific targets are selected through a process termed as SELEX (Tuerk 1990b) which is based on chemically synthesized oligonucleotide forming a combinatorial library. The latter consists of multitude of single-stranded DNA (ssDNA) or RNA fragments with different sequences ($\sim 10^{14}$–10^{16}) (Marty and Hayat 2014). This selection process for emerging pathogenic microorganisms has two critical steps: (1) targets screening and (2) separation of the binding sequences. Briefly, the selected aptamers are initially incubated with the target(s) of interest (i.e., pathogens) followed by separating the high-affinity aptamers from that of unbounded target(s). Next, the eluted aptamers are amplified using polymerase chain reaction (PCR) to get an enriched pool for more selection rounds. These aptamers are cloned, sequenced, characterized, and may be manipulated to achieve aptamers with desired characteristics (Fig. 1) (McKeague and Giamberardino 2011; McKeague and Giamberardino 2011; Wang et al. 2012).

The ultimate goal of modifications is to select aptamers with specific properties such as efficiency, rapidity, and less labor. Based on aptamer properties and screening methods, the SELEX process has been improved to get high-affinity and selectivity aptamers via improving selection, amplification, and pool enrichment monitoring (Kim and Gu 2014). Other changes include target immobilization and nucleic acid library expension (McKeague and Derosa 2012). For oligonucleotide stability and resistance against nuclease enhancement, several SELEX protocols have been developed such as genomic SELEX or cDNA–SELEX (Zimmermann et al. 2010), covalent SELEX (Kopylov and Spiridonova 2000; Spiridonova 2014; Spiridonova et al. 2014), deconvolution SELEX or subtractive SELEX (Torres-Chavolla and Alocilja 2009), photo SELEX (Cho et al. 2004), and multistage SELEX (modified chimeric SELEX) (Wu and Curran 1999) for rationalization of starting pool. Counter SELEX or negative SELEX protocol in aptamer is for selectivity enhancement (Xi et al. 2014). For target applicability, expression cassette (Martell et al. 2002), TECS-SELEX (Ohuchi et al. 2006), whole-bacteria SELEX (Torres-Chavolla and Alocilja 2009), mirror-image SELEX

(Klussmann et al. 1996), blended SELEX (Kulbachinskiy 2007), toggle SELEX target-switching (Hamula et al. 2006), whole-cell SELEX (Park et al. 2014b), complex target SELEX (Chen 2007) are more promising. Current emerged techniques such as HTS or automated SELEX (Huenniger et al. 2014; Lu et al. 2014), Non– or NECEEM–SELEX (Ashley et al. 2012; Yun et al. 2014), CE-SELEX (Kasahara et al. 2013), FluMag-SELEX (Stoltenburg et al. 2005a), microfluidic SELEX (Lin et al. 2014), and in silico SELEX (Nonaka et al. 2013; Savory et al. 2013) have improved efficiency of designed aptamers in both binding affinity and high throughput in tertiary structure prediction, thermodynamic, and aptamer–target model (Das et al. 2010). Despite all these enumerated SELEX techniques, some whole-cell-selected aptamer candidates would have high affinity but poor specificity (Hamula et al. 2008). Thus, in most current used techniques for pathogens detection, accurate counter selection and FACS sorting (FACS-SELEX) steps are crucial for effective aptamer candidates (Fig. 2) where bound sequences are perfectly separated from unbound ones. Moon and others reported that the use of cell sorting followed by FAM-labeling of aptamer candidates improved the affinity up to a Kd of 3.49 ± 1.43 nM (Moon et al. 2014).

SELEX protocol modification has been acquired also in optimizing PCR amplification towards precise number of rounds franking primer sequences to the high-affinity sequences of oligonucleotides. In this novel in vitro selection, fixing primer sequences allows to restrain a double-stranded structure of oligonucleotides. It allows primerless selection to prevent the primer binding sequences to incorporate into the target oligonucleotide-binding motif (Florian Jarosch 2006). Additionally, the use of unnatural bases (library modification) using expanded genetic alphabet of oligonucleotides contributed to a >100-fold affinity DNA aptamers as compared to the natural ones. Kimoto and colleagues have designed a DNA aptamer with four natural nucleotides (dNTPs) and unnatural nucleotides (dDsTPs, dPx, and dPaTP) recognizing the human proteins such as signal protein vascular endothelial cell growth factor-165 (VEGF-165) and interleukins (interferon-γ (IFN-γ) with binding affinity of Kd values of 0.65 pM and 0.038 nM, respectively) (Kimoto et al. 2013). In addition, the use of IsoG, IsoC, and other unnatural base pairs combined to the natural ones functions as third base pair. These modifications are prominent to high efficiency and selectivity in new modified SELEX process through hydrogen bonding principle and their inotropic properties towards aptamers (Fig. 2); however, to date, few reports have been applied to affinity selection methods. Furthermore, polynucleotide library with modified polynucleotides such as 2′-amino pyrimidines, 2′-fluoro pyrimidines, 2′-O-methyl nucleotides, boranophosphateinternucleotide linkages, 5′-modified pyrimidines, 4′-thio pyrimidines, phosphorothioate inter-nucleotide linkages providing high stable and affinity, nuclease resistant,

Fig. 1 Schematic illustration of SELEX process with slight modifications for high affinity aptamer generation
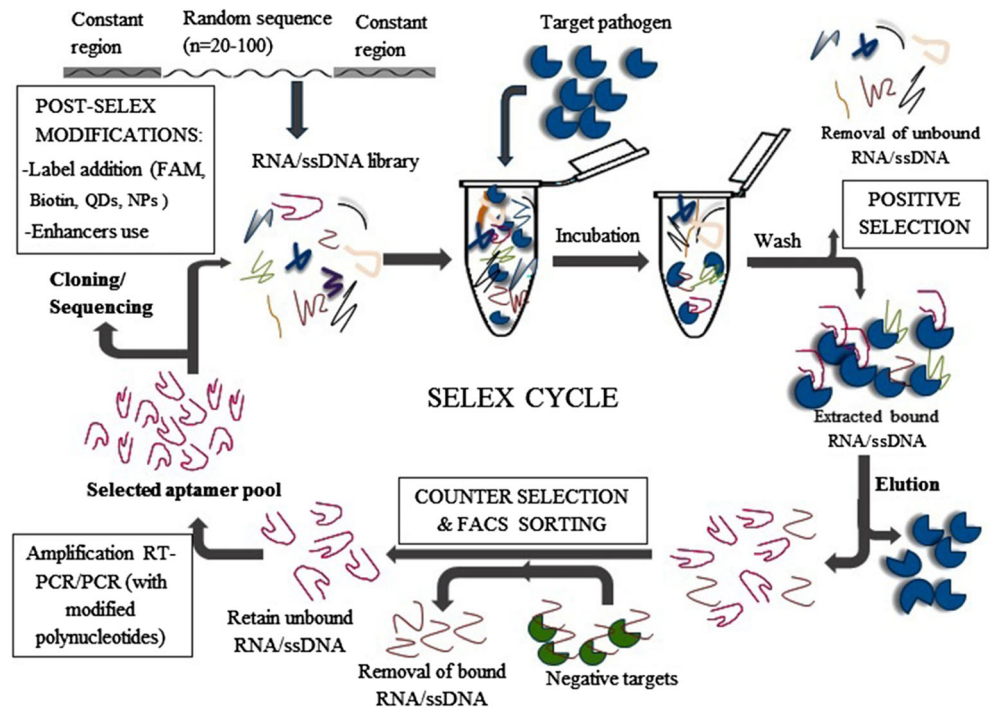

and most importantly SELEX-compatible and cheap synthesis aptamers (Keefe and Cload 2008) showing their use in both therapeutic and diagnosis of diseases and detection of tiniest emerging foodborne pathogens.

Food Pathogen Detection with Aptamers

Foodborne illnesses and outbreaks are commonly caused by bacteria. Typically, symptoms of infection do not appear until

Fig. 2 Illustration of improved process of FACS-SELEX process for high affinity aptamers selection: Classic method: contains ten rounds of SELEX and only one FACS sorting. This procedure gives aptamers with adequate binding affinity (*Aptamer A*). More current procedure of FACS-SELEX provides aptamers with high binding affinity to its targets with less SELEX rounds (*Aptamer B*). In this process, the oligonucleotide library is fluorescently labeled and then preincubated with a mixture of different kinds of targets. Aptamers can be separated by flow cytometer depending on affinity. The aptamers bound only to the cells of interest are then eluted, purified, and amplified
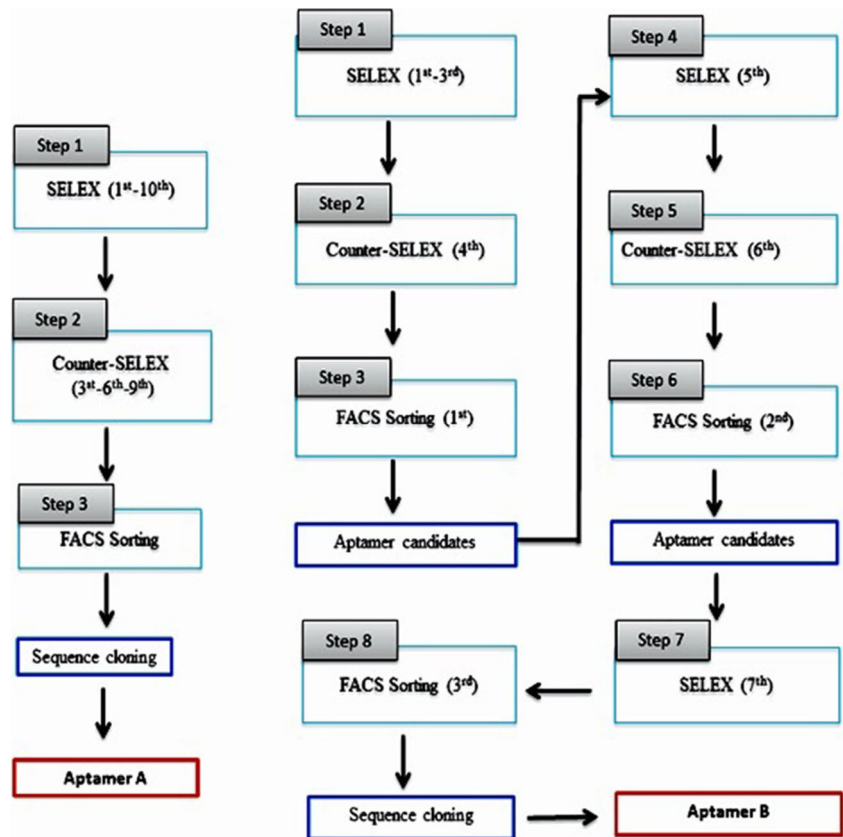
~12–72 h after food ingestion. Thus, it is a need of hour to detect pathogens timely, rapidly at very low levels in complex food matrices to lessen the risk of illness and to avoid product recalling. To overcome these challenges, a number of aptamers and nanomaterial-labeled aptamers in conjunction with electrochemical transduction output with high potential for bacteria detection have been developed (Marty and Hayat 2014; McKeague et al. 2011). Nanoparticles such as silver, gold, and quantum dots are utilized as novel tools as compared to immunoassay-based and chromatography-based methods, because they provide fast and reliable detection approach for pathogens that pose serious concerns to humans. The underneath described putative aptamers could be considered as potential approaches for detection of common food pathogens namely *Salmonella* sp., *Staphylococcus aureus Bacillus* sp., *Campylobacter* sp., *Escherichia coli*, *Shigella* sp., *Listeria monocytogenes*, and *Streptococcus pyogenes*.

## Salmonella Species

They are the most common foodborne causative agents of parathyroid fever, typhoid fever, and salmonellosis with multidrug resistant strains.The classic microbiological detection of *Salmonella* genera requires standard steps of cultivation such as preenrichment, selective enrichment, and selective differential media plating which are tedious, expensive, complex operations and require long time of incubation. DNA aptamers has demonstrated binding specificity to most of the genera. Dwivedi and others developed selected an aptamer recognizing *S. typhimurium* through magnetic capturing. In this study, primers were prelabeled and obtained DNA aptamer pool has been sorted using FACS followed by biotinylation. It displayed less cross-reactivity to other foodborne pathogens and possessed good Kd of 1.73 ± 0.54 μM detected in low sample of 290 μL containing 10^2–10^3 cfu/mL (Dwivedi et al. 2013). The use of FACS shows a remarkable alternative in production of high affinity and pure aptamers recognizing specifically cell membranes compartments (Jay 2013). In addition, an electrochemical biosensor was developed using a *Salmonella*-specific recognition aptamer biosensor. It is made with a glassy carbon electrode@graphene oxide and aptamer-functionalized gold nanoparticles (AuNPs). This aptamer ssDNA sequence could be linked to electrode via thiol (–SH) groups and AuNPs using three-electrode systems where *Salmonella* is incubated to generate current between electrodes by decreasing the electrolyte that resulted in high resistance. The selected aptamer has high specificity and selectivity with detection range of 2.4–2.4×10^3 cfu/mL that was achieved and LOD of 3 cfu/mL (Ma et al. 2014) Fig. 3b.

For *S. typhimurium*, the RNA aptamer-based ligands were specifically identified through SELEX. The isolated and characterized RNase-resistant RNA aptamer was targeting outer-membrane proteins (OmpC) with high specificity and affinity (Kd = 20 nM). The selected aptamer binds only to *S. typhimurium*, and none of other related bacteria used in counter selection bounds to it (Han and Lee 2013). However, resilient efforts were made in whole-cell SELEX modification to increase the success rate. Four important steps summarize this modification: (1) incubating the ssDNA library with live *Salmonella* sp. at ambient temperature for aptamers binding, (2) separating the bound and unbound ssDNA from target in centrifugation process, (3) amplification of the bounded ssDNA (in this process, ssDNA is concentrated by PCR-Ultracel), and (4) finally, ssDNAs are sorted, cloned, sequenced, and then individually characterized according to their binding affinity for *Salmonella* cells using flow cytometry. Through abovementioned process, a potential ligand aptamer (5′-ACGGGCGTGGGGGCAATGCT GCTTGTAG CCTTCCCCTGTGCGCG-3′) has been selected for capturing *S. typhimurium* (Moon et al. 2013). Other aptamer candidates such as 5′-TATGGCGGCGTCAC CCGACGGGACTTGACATTATGACAG-3′ and GAGGAAAGTC TATAGCAGAGGAGATGTGTG AACCGAGTAA have also been selected after the seventh round of SELEX and exhibited low LOD of 10–40 cfu/mL. These aptamers are DNase resistant which enhance specificity towards outer membrane proteins (OMPs) and high affinity detection when using spectrometry (Raghavendra Joshi 2009). Another selection of *S. typhimurium* with high-affinity ssDNA aptamer was designed based on bacterium-based SELEX. The sequence (5′-FAM-ATAGGAGT CACGACGACCAGAAAGTAATGCCCGGTAGTTATTCA AAGA TGAGTAGGAAAAGATATGTGCGTCTACCTCTT GACTAAT-3′) showed a high affinity for *S. typhimurium* ($Kd = 6.33 \pm 0.58$ nM). With this aptamer, the detection range of 50–10^6 cfu/mL and LOD of 5 cfu/mL were obtained (Duan et al. 2013c). These approaches could be employed to develop an easy, rapid, and sensitive methodology for other closely related pathogen detection in food(s) as only aptamer–AuNPs based colorimetric method can detect 10^6 cfu/mL (Moon et al. 2014).

Recently, aptamer-combined AuNPs is ever-increasing in the most successful strategy in pathogen detection. Yuan and others used biotinylated aptamer (1)/bacterial/aptamer (2)-AuNPs sandwich system with 5′-biotin-C6-TATGGCGG CGTCACCCGACGGGGACTTGACATTAT GACAG-3′ and 5′-SH-TATGGCGGCGTCA CCCGACGGGGACTT GACATTATGCAG-3′ for *Vibrio parahaemolyticus* and *S. typhimurium* aptamer-AuNPs, respectively. The aptamers were immobilized on the well's surface of the microtiter plate via biotin–streptavidin binding accompanied by silver enhancement for binding specificity, sensitivity, and stability. The optimal addition of silver enhancer solution provides visual differentiation between *S. typhimurium* and other foodborne bacteria through signal intensity. With this method of gold-labeled silver staining, the LOD of *S. typhimurium*
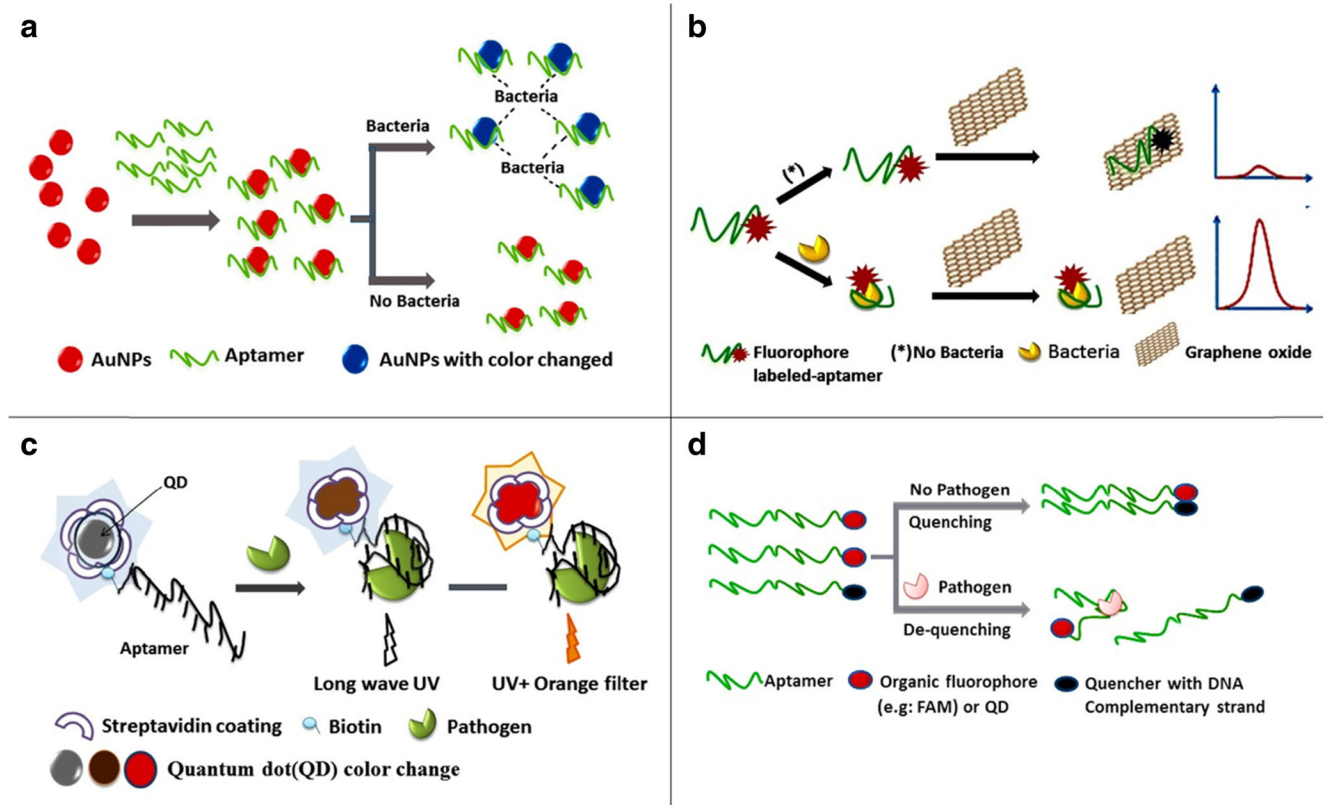
Fig. 3 Aptamer enhancers: Aptamer-functionalized gold nanoparticle (AuNP). AuNPs possess a strong surface plasmon resonance band, which has strong distance-dependent properties. When AuNPs bind to targeted sites and come into close proximity with other AuNPs, they change the refractive index of the surrounding medium, resulting in a color change from *red to purple*. **a** Graphene oxide-SELEX: The ssDNA library is preincubated with AuNPs followed by adsoption on GO via to π–π bonding. **b**. The DNA capture element (DCE) system quenching and dequenching. This process consists of fluorophore (e.g., QDs, FAM,)-labeled starting ssDNA library through covalent linkage at the 3′ end and a bead covalently linked to the 5′ end, and the complementary strand aptamer has a quencher covalently linked to the 5′ end. The two strands of DNA are *annealed together*, and the quencher quenches the fluorescence of the fluorophore. The presence of targets triggers the DCE system fluctuation resulting in the double strands denaturation which gives fluorescencer. **c** Illustration of capture aptamer and UV light requirement to immobilize the capture aptamer. Colloidal gold or quantum dot is used as reporter conjugate. **d** The aptamer multiplex system may involve digoxigenin-5′ reporter aptamer-3′-biotin–streptavidin colloidal gold or Q-dot conjugates, amino-capture aptamers immobilized with UV light for colorimetric observation. The intensity of the dots increases proportionally with targets

reached 7 cfu/mL (Yuan et al. 2014) which depicts the prospective utilization of combinatorial techniques in improving the microbial visual detection (Fig. 3d).

Few years back, we used aptasensors and dual fluorescence from green QDs and red QDs nanoparticles as donors and amorphous carbon nanotubes as acceptors could give the LOD of 25 and 35 cfu/mL in food sample for *Vibrio parahaemolyticus* and *S. typhimurium*, respectively (Duan et al. 2013d). Interestingly, this method provided improved simultaneous and visual detection of pathogens based on QDs fluorescence which is linearly proportional to the target concentration. Furthermore, a high affinity aptamer (5′-ATGGACGAATATCGTCTCCCAGTGAATT CAGTCGGACAGCG-3′) has been screened using whole-cell SELEX conjugated carbon nanomaterials to detect the *S. paratyphi* A and achieved $Kd = 47 \pm 3$ nM. Additionally, a SWCNTs@ DNAzyme- aptamer combination was designed for this pathogen and this complex has self-aggregation in the presence of specific target. A linear range of 10^3-10^7 cfu/mL was attained with a LOD of 10^3cfu/mL (Ming Yang 2013). More currently, a remarkable combinatorial technique of *S. typhimurium* detection has been published. Here, A Surface-enhanced Raman spectroscopy (SERS) substrate bearing Au@AgNPs combined with 5′-SH-aptamer 1 for target cells capture followed by aptamer 2 modification using X-rhodamine (ROX) (recognition element and SERS reporter). *S. typhimurium* specifically bind with the aptamers to form Au@AgNPs-aptamer1-target-aptamer2-ROX sandwich-like complexes. This tremendous technique results a rapid and high sensitive detection with a linear range of 15–1.5 × 10^6 cfu/mL with a LOD 15 cfu/mL(Duan et al. 2016). Though many techniques have been discussed, this technique promises potential use in foodborne pathogens detection in terms of LOD and feasibility.

Staphylococcus aureus

It is a pathogen commonly found in food. It secretes a mixture of enterotoxins known as staphylococcal enterotoxins A and B (SEA and SEB). (Bruno et al. 2010; Bruno JG 2002; Tombelli S 2007). For these toxins, aptamer targeting SEB1 has been selected for SEB (DeGrasse 2012b). Though its high affinity and selectivity together with other several ssDNA aptamers to SEB were described, their aptamer sequences are not implemented, limiting their utilization. For *S. aureus* SEA, a method based on primarily antibody dependent was replaced by aptamers that bind to target with high affinity and selectivity. Those aptamers were selected and generated in vitro by a 12-round selection process based on magnetic separation ($Kd = 48.57 \pm 6.52$ nM). This aptamer shows more reliable results consistent with previously reported *S. aureus* SEA detection having a LOD of 8.7×10^{-3} μg/mL (Huang et al. 2014) with low cost and high affinity compared to its antibody-based counterpart which is challenging and laborious.

For whole-cell-based SELEX, 10^7 *S. aureus* have been incubated for aptamer selection followed by isolation of bound aptamers. In the counter-selection process, aptamers with specific binding were incubated with structurally related negative targets (10^8 *S. epidermidis*) and highly specific aptamers were amplified and purified. The selected ssDNA was again incubated with new *S. aureus* to start next SELEX round(s) and after eight rounds cloned and sequenced. Likewise, a panel of ssDNA aptamer specific to *S.aureus* was obtained by a whole bacterium-based SELEX and applied to probing *S. aureus*. Here, 11 sequences from different families were selected for further characterization by confocal imaging and flow cytometry. The *Kd* values obtained for a single candidate aptamer were in the nanomolar range and showed a high specificity (Cao et al. 2009). Recently, combinatorial assays for simultaneous detection of three pathogenic bacteria was developed using multicolor up conversion nanoparticles (UCNPs) as luminescence labels coupled with aptamers as the molecular recognition elements (Wu et al. 2014). Other simple and fast method based on a polydimethylsiloxane (PDMS)/paper/glass hybrid microfluidic system integrated with aptamer-functionalized graphene oxide nanobiosensors for simple, one-step, multiplexed pathogen detection has been developed. This method has been simultaneously used in detection of two infectious pathogens *S. aureus* and *S.enterica* (Zuo P 2013). Chang et al. performed two ssDNA aptamers with high affinities and specific against *S. aureus* ($Kd = 35$ and 129 nM). This *Kd* was improved to 3.03 and 9.9 nM which demonstrated the detection of single *S. aureus* cell in 1.5 h and promised the constructed aptamer to be used in food safety for *S. aureus* detection (Chang et al. 2013b). Recently, chemically modified aptamer-functionalized grapheme oxide combinatorial technique has been developed able to detect a single colony-forming units per milliliter. In this technique, graphene works

as a transducer layer of aptasensor while the aptamer is a sensing body. The modified graphene showed less noise compared to non-modified in detection of challenging strains like *S. aureus* (Hernández et al. 2014). This study demonstrated a high sensitivity of combination than either bare-used aptamers or nanomaterials in recognizing *S. aureus*. This technique promises potential use in foodborne pathogen detection. Though its high affinity and selectivity together with other several ssDNA aptamers to SEB were described, however, their aptamer sequences are not implemented in lieu or as alternative of antibodies, limiting their utilization (DeGrasse 2012a).

Bacillus Species

Bacillus anthracis is a rod-shaped, Gram-positive bacteria able to form endospores. It causes disease to both human and livestock owing to its immune system escaping mechanism and causes high fatality rate derived from its high load in food such as hot chocolate, rice, mashed potatoes, and turkey and meat loafs (Drobniewski 1993; King et al. 2007). Etiologically, it is known as major cause of anthrax in livestock and toxicity in human acquired from its membrane surface protein capsule namely poly-D-gamma-glutamic acid and lethal factor endopeptidase that hydrolyze mitogen-activated protein kinase kinases (virulence part). Recent novel strategies are able to differentiate between the *Bacillus* strains although some setbacks occur in detection of *B. anthracis* especially phenotypic and genotypic resemblance within bacillus genera thus high possibility of getting the false results. For example, *B.thuringiensis* and *B.cereus* are closely related with *B. anthracis* with only two dissimilarities: (1) two virulence plasmids in *B. anthracis* and insecticidal toxins coding plasmids in *B. thuringiensis* (Warda et al. 2016). Gram-positive bacteria *B. cereus* is responsible for causing severe nausea, vomiting, diarrhea, and other similar foodborne illness that has the threshold for onset the disease(s) is >10^6 *B. cereus*/g in food produce (Helgason E 2000), and yet, no putative aptamer-conjugated essay was reported. As *B. thuringiensis* is also injurious to human, a DNA aptamer 5′-CAT CCGTCACACCTGCTCTGGCCACTAACATGGGG ACCAGGTGGTGTTGGCTCCCGTATC-3′ was specific for *B. thuringiensis* whereby combination of 60 base aptamer to fluorescent zinc sulfide-capped cadmium selenide quantum dots (QD) detect *B. thuringiensis* (*B. cereus* closely related bacteria). During this testing, several controls with non-functionalized QDs and without spores were verified and validated to measure QDs specificity to the spores and the fluorescence attained the LOD of 10^3–10^4 cfu/mL (Ikanovic et al. 2007; Torres-Chavolla and Alocilja 2009). For specificity purposes, the detection of *B. globigii* spores (*B. subtilis* vs. *B. niger*) can differentiate *B. thuringiensis* from *B. globigii* at concentrations above 105 cfu/mL (Lim et al. 2010) (Fig. 3b). Additionally, the use of Raman spectroscopy

combined to the single-walled carbon nanotube (SWCNT) generates high affinity oligonucleotides for *B. anthracis* detection. A complementary DNA to the target DNA is added to DNA-SWCNT complex resulting hybridization (Bansal et al. 2013).

To date, SELEX is the most often used process in aptamer selection for various purposes including food safety. However, during the selection process, it requires high amounts of target molecules. Therefore, as a possible alternative, another sensing system called DNA capture element (DCE) is used to detect scarce pathogens (Tuerk 1990b). *B. anthracis*, Shiga toxin, botulinum neurotoxin (BoNT), and *Francisella tularensis* DCE systems have been developed from their aptamers (Mathew et al. 2015; Tuerk 1990b). In DCE approach, the target interacts with dsDNA and magnetic separation technology followed by its denaturation (quenching and dequenching system) resulting fluorescence signals (Fig. 3c) (Fan et al. 2008). The resulted target-bound ssDNA complex can be separated later by random ssDNA magnetic beads. The use magnetic beads requires very small amount of target during ssDNA/target separation and helps in signal amplification. The bacillus spores were used as targets of DNA aptamer during screening, and 79 DNA fragment sequences from 13 different classes of DNA aptamers were obtained by 18 cycles (Wang et al. 2012). A DNA aptamer (5′-CATCCGTCACACCTGCTCTGGCCACTA-3′) obtained against spores of *B.anthracis* strain has paved the way to develop more sophisticated aptamer-magnetic bead–electrochemiluminescence (AM-ECL) sandwich technique with high sensitivity of detecting <10 *B. anthracis* spores (Xu et al. 2011). The binding of quantum dot (QD)-labeled aptamer to *B. cereus*, *B. anthracis* spores, could be applied to target similar pathogens (Yang et al. 2013).

Campylobacter jejuni

Campylobacter jejuni is a common food poisoning bacterium and mainly found in raw meat, raw milk, and cross-contaminated foods. When ingested with contaminated food, it colonizes the intestine wall and produces enterotoxins. Dwivedi and others performed cell-SELEX on live C. *jejuni* by applying combinatorial library of FAM-labeled ssDNA on whole-cell SELEX method. Here, ssDNA molecular recognition elements (MREs) are used followed by FACS sorting targeting *C. jejuni* A9a. These aptamers showed high affinity and specificity against the target with relative high dissociation constant of 292.8 ± 53.1 nM (Jaykus 2010; Dwivedi et al. 2010b). Furthermore, Stratis-Cullum and others performed a CE-SELEX to evaluate the aptamer specific for dead *C. jejuni* with qualitative capillary electrophoresis immunoassay and the obtained LOD was 6.3×10^6 cells/mL (Stratis-Cullum et al. 2009). Aptamer-linked QDs have been developed and used in a sandwich assay in conjugation with magnetic beads for the

detection of *C. jejuni* at levels of between 2.5 and 10–50 cfu/mL in buffer and food matrices, respectively (Bruno et al. 2009). However, currently, it was proved that biotinylated magnetic bead-based aptamers followed by qPCR detection of *C. jejuni* in sample volume range of 300 µL to 10 mL. This combination targets the glyA gene and is species specific with clear exclusion of closely related genera (Suh et al. 2014b). The qPCR complemented with biotinylation properties such as fast on rate, specificity, affinity, and nucleic acid stabilization. The principle behind consists of aptamer conjugation to its target with donor and acceptor microbeads. After interaction, there is fluorescent signal production which could be read by fluorescence reader. This automated biotinylated magnetic bead qPCR-based aptamers can simultaneously detect targets with high affinity and in a short time.

Escherichia coli

Escherichia coli have enterotoxigenic effects and potentially contaminate food and water (Castro-Rosas et al. 2012). Detection of *E. coli* O157:H7 is of high priority in food surveillance as this Gram-negative bacteria cause infection which may lead to hemorrhagic diarrhea and kidney failure (Bieke Van Dorsta 2010; McKeague and Giamberardino 2011). Sensitive methods are of great importance as the infectious concentration can be as low as 10–100 cfu/mL. Several DNA and RNA aptamer-based platforms have been developed for several strains of foodborne *E.coli*. One example is an aptamer-modified SWCNT in both field-effect transistors and label-free potentiometric measurements to provide rapid and reusable biosensors. For effective potentiometric aptamer-based biosensor, a FRET-based assay using a DNA aptamer for nonpathogenic *E. coli* CECT 675 strain is used as a model organism substitute for pathogenic *E. coli* O157:H7. The SWCNT works as ion–electron transducers, and aptamers are covalently immobilized on it as a biorecognition elements. Aptamers interact with target(s) with subsequent electrical potential changes that enables quick identification and detection of the target. As a result, 10^4 cfu/mL can be detected selectively and this biosensor can be built and regenerated easily with a low LOD in complex samples (Zelada-Guillén et al. 2010). Furthermore, aptamer-functionalized SWCNT field-effect transistors have also been reported for the detection of *E. coli* DH5α strain (So et al. 2008). However for endotoxins, producing *E. coli* strains, only *E. coli* O111:B4 endotoxin is reported. Here *E. coli* cells *or* lipopoplysaccharide (LPS) are used as target for selecting specific aptamer and showed high affinity and selectivity when analyzed by a colorimetric enzyme-based technique (Bruno et al. 2008).

Other nanomaterials in conjunction with aptamer-based biosensors for *E. coli* detection were developed. An example is a Fe_2O_3–AuNPs electrochemical DNA biosensor. This DNA biosensor forms a sandwich complex made of

recognition element on Fe_2O_3@AuNPS, reporter@HRP enzyme, and the target. After *E. coli* capture, the induction of magnetic field activates magnetic separation of complexes leading to HRP catalysis resulting in detectable signals. The LOD of 5 cfu/mL was achieved without requiring PCR amplification of target DNA (Li et al. 2011), though it required long-time incubation period (Fig. 3a). This assay is reliable; however, its detection sensitivity is of concern in terms of working time. Therefore, a DNA aptamer–quantum dot lateral flow test strip can improve the detection time and provide colorimetric results for more common foodborne pathogens. At the presence of targets, the high-affinity selected aptamers stabilize nanoparticles and the latter act as signal transducer elements via their aggregation leading to pinkish visible color change from red (Bruno 2014).

Currently, Kim and others detected *E. coli* strains that used fluorescence-tagged aptamer cocktails and found this can boost signals than single aptamers. The use of three various aptamer cocktails, each labeled with FAM, is able to recognize one particular bacterium and target the outer membrane compartments. Compared to single aptamers, the cocktail enhances sensitivity and fluorescence signal simultaneously with cumulative influence. This method is attractive because it can reduce the LOD from 6778 to 3.7×10^2 cfu/mL of single aptamer and multiple ones, respectively (Kim et al. 2014). However, due to outer cell surface complexity, molecular level understanding of how these aptamers bind on *E. coli* strains is still unknown. Using, the same aptamers could bind to cell surface components competitively followed by fluorescence emission; therefore, two or more different aptamers could be assumed to bind non-competitively (Kim et al. 2013c).

Shigella Species

Shigella dysenteriae is a Gram-positive, facultative intracellular and shigellosis, epidemic bacillary dysentery causing bacterium. It is the dominant cause of and poses a major public health problem worldwide (Kotloff et al. 1999; Niyogi et al. 2005). People having lack of access to adequate clean drinking water, sanitation of environments, and dwelling in slums are especially at higher risk of *S. dysenteriae's* contagiousness causing a million of deaths each year (Sur et al. 2004). Few techniques have been used alongside with classic conventional culture. Immunomagnetic detection of *S. dysenteriae* type 1 and *S. flexneri* serotypes in feces has been used targeting *Shigella* sp. O-antigen (Islam et al. 1993) and lipopolysaccharides (Carlin and Lindberg 1987) and PCR method with primers targeting invasion plasmid antigen H gene sequence(*ipaH* gene) (Thiem et al. 2004). Duan and others used cell-SELEX to identify ssDNA MRE specific for *S. dysenteriae*. The best candidate MREs (CGGAACTA GCGTTTAAATG CCAGGACTGAAGTAGGCAGGG) had demonstrated probable low binding affinity

($Kd = 23.47 \pm 2.48$ nM) targeted bacteria. Fluorescent-based assay detection was carried, and results showed a LOD of 50 cfu/mL. This aptamer could be employed for the capture and subsequent detection of *S. dysenteriae* from complex sample matrices and food (Duan et al. 2013b) and bears strong merits over market-dominated antibody and PCR-based technique. However, it is the only disclosed aptamer and no available aptamer-combinatorial technique used in detection of this pathogen. Furthermore, other *Shigella* sp. like *S. sonnei* is currently causing foodborne disease with shiga toxin production (Lamba et al. 2016). Our group developed a more simplified *S. sonnei* sandwich detection system. Selected aptamers Sp1 and Sp2 were analyzed by FACS and found to reach the sensitivity of 5.980 ± 0.835 and 14.32 ± 2.19 nM, respectively. Then, both aptamers were used in a sandwich manner to capture the target. The LOD of 30 cfu/mL were achieved (Gong et al. 2015). To date, few available combinatorial aptamers are reported. At this point, aptamer–ELISA has been used with antibody as a recognition element of the target (Elham Masoudipour 2011). However, this combination approach is tedious, expensive, and requires professionalism. Therefore, it is of utmost importance to design alternative combinatorial approaches for improved detection of this pathogen.

Listeria Species

It is pathogenic bacteria Gram-positive, facultative anaerobes, rod-shaped bacteria. Its genus *Listeria* contains eight non-pathogenic species (*L. marthii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, *L. rocourtiae*, *L. fleischmannii*, and *L. weihenstephanensis*) and two pathogenic species (*L. monocytogenes* and *L. ivanovii*) which cause infection to human as well as animals (Kuenne 2010). Motility, catalase positive, and virulence are its unique features; however, little is known about its virulence. They are dispersed into the low temperature environs and most frequently in the soil, water, sewage, and foodstuffs (L. Somer 2003). The European Union in collaboration with FDA has applied a zero tolerance on *L. monocytogenes* in ready-to-eat food products as its rate of death is beyond *C. botulinum* and *Salmonella* spp. (CDC 2013). Although listeriosis is rare, still serious illness predominantly affects sensitive populations, i.e., infants, elders, and immunocompromised patients (Kathariou 2002). The ssDNA aptamers with binding affinity to *Listeria* spp. have been selected using whole-cell SELEX method, and aptamers with binding affinity were analyzed by flow cytometry. A ssDNA aptamer (LM6-116) 5′-TACTCGTTATTTCGT AGCACTTT TCCCCACCACCTTGGTG-3′ binds various cell surface components (Suh et al. 2014a).

The use of SELEX to an ssDNA-conjugated biotin library is currently applied in aptamer selection and characterized with flow cytometry. The selected aptamer displayed a good binding affinity to *L. monocytogenes*. The selected aptamer 5′-

GAGGGAAGAAGGGCCAGCACAGATCAGATCAA TCGCTCCG-3′ conjugated to magnetic beads and when used in a combined aptamer magnetic capture (AMC)-qPCR assay: the pathogens were detected at concentrations <60 cfu/500 μL buffer in the presence of non-Listeria mixtures bacteria with a capturing efficiency of 26–77%. This method shows a high LOD 2.7 and 4.8 log cfu/L (Suh and Jaykus 2013). In another SELEX-based technique, an aptamer-A8 (5′-ATCC ATGGGGCGGAGATGAGGGGGAGGAGGGCGGGTAC CC GGTTGAT-3′) specific for internalin A (L. monocytogenes invasin protein) was used in the fiber optic sensor together with antibody in a sandwich format for detection of L. monocytogenes. This selected aptamer was very selective to L. monocytogenes with quantitative detection of ~10^3 cfu/mL (Ohk et al. 2010) which is of concern due to the use of bare aptamer. For enhanced detection, a whole-bacterium SELEX strategy and fluorescence-based recognition for rapid detection of L. monocytogenes was reported. Here, the following sequence TGGGAGCTCAG AATAAACGCTCAACTTTGTTCTTCTTGCTTTT TTTTTCTTTTTTTGTTCGACATG was modified with digoxigenin for direct fluorescent observation after combining with targeted bacteria and a fluorescent microscopy was used for direct observation of digoxigenin–aptamer binding to the target L. monocytogenes with much higher specificity (Liu Guo-qing 2014). Recently, Liu and others have shown that eight rounds of selection give ssDNA molecular recognition elements (MREs) specific for L.monocytogenes with high specificity up to Kd value of 60.01 nM (Liu et al. 2014). Moreover, we achieved the whole cell in vitro selection on L. monocytogenes and selected aptamer showed a considerable affinity (Kd = 48.74 ± 3.11 nM). A fluorescent binding bioassay was developed to confirm the high specific binding of these MREs towards targets demonstrated a LOD of 75 cfu/ mL (Duan et al. 2013a). In summary, both bare and modified aptamers have significantly contributed in improved detection of pathogens and at the same time noticeably reduced the cost incurred for detection method(s) when compared to antibodies. Despite the advantage(s) of each approach, available techniques for this pathogen are only fluorophore–aptamer-based combination rather than the use of other enhancers.

Streptococcus Species

Streptococcus species are spherical, Gram-positive bacteria belonging to Firmicutes phylum and Lactobaliales order. Many species are facultative anaerobes, catalase-negative, not motile, and occur in chains or pairs with ability to adjust at low temperature (<7 °C). Medically, Streptococcus species are classified depending on their hemolytic characteristics group namely alpha and beta hemolysis. They have the ability to oxidize hemoglobin iron or destroy completely red blood cells, respectively. Depending on antigenicity and physiological characteristics, Streptococcus genus is grouped into A, B, C, D, F, and G; and among them, groups A Streptococcus (GAS, with 40 antigenic species) and D (Enterococcus) can be transmitted via food. S. pyogenes possess ten major M-type surface proteins (M1, M2, M3, M4, M6, M11, M12, M28, M77, and M89) constituting its major virulence factor (Nitzsche et al. 2015). They are able to cause various infections to all individuals such as impetigo, pharyngitis, and other serious infections like necrotizing fasciitis, sepsis, and frequent GAS that may cause post-infection autoimmune diseases such as rheumatic fever, glomerulonephritis, and heart diseases which lead to high mobility and mortality (Walker et al. 2014). To date, immunodiagnosis and classic diagnosis still dominate despite tedious procedure of stool, saliva, swab, blood, and contaminated food samples culture, depicting the need of simple and fast detection techniques of these pathogens (Kim et al. 2013b). Though aptamer framework contribution in recent year increased, apparently few GAS species are studied intensely. It has been reported a combinatorial technique sensor consisting of immobilization of aptamer on SWCNTs via p–p stacking bond of ssDNA which is serially connected to Au electrode and piezoelectric quartz crystal. This complex is detached when the immobilized anti-GAS aptamer react with GAS resulting frequency shift response because aptamer binding force to SWCNT is lower than its binding on GAS. The detected impedance spectra increased precisely to aptamer–GAS detachment with 3×10^2 to 3×10^6 cfu/mL concentration and the LOD of 12 cfu/mL in 40 min (Shi et al. 2014). However, this technique obviously could not detect targets when aptamers are dispersed or scarce since the frequency shift increased in a low range of aptamers concentration from 1 to 10 μM where the increase of aptamer concentration to 12 μM lowered the frequency shift due to their accumulation which can hinder the GAS recognition. Currently, Hamula and others developed targeted ten common and other ten non-common M-type S .pyogenes. Bacteria were incubated with 80 nucleotide DNA libraries, and other process follows standard SELEX procedure mediated with FACS to assess the binding capacity of aptamer pool and 5′ FAM labeling for visual detection. The Kd = 4 to Kd = 86 affinity range was achieved (Hamula et al. 2011). The same group improved S. pyogenes targeting aptamers with modification in standard SELEX. Special forward primers are designed to contain polyadenines linked to it via triethylene glycol spacer that plays a critical role in gel electrophoresis separation of both primers to the PCR product. The reverse primer was fluorescently labeled with 5′ FAM to allow FACS step (Mayer et al. 2010). During incubation, selected aptamers were incubated with S. pyogenes, and tRNA and BSA were added in incubation buffer to increase ssDNA recognition of cell target. The modified PCR sequences, counter-selection using non-M-type cells, and finally, FACS analysis improved binding of M-type 11 protein of

S. pyogenes with a $Kd = 7 \pm 1$ nM (Hamula et al. 2016). Though this binding affinity can be achieved by immunoassay-based technique, this technique is less laborious and comprehensive. Moreover, these aptamers can be fixed to solid surface for aptamer array development used in M typing or whole-cell detection of food or clinical samples. However, to date, no research has been reported for the other predominant M types of GAS, depicting the need to develop a reliable technique of detection with further modification(s) in aptamer–immunoassay combination where fluorescently labeled aptamer or antibody recognize the non-variable M-type protein of the bacteria and/or its C-terminal residues in a sandwich manner.

Conclusion and Future Directives

Aptamers were shown to be an attractive alternative to immunosensors and enzyme-based sensors for microorganisms monitoring. Those oligonucleotides are proving to be effective molecular recognition probes of high priority in food and drug quality and safety testing, disease diagnosis. etc.

They are relatively stable with ease synthesis and modification. The aptamer stability is of great importance for bacteria detection in food samples. Therefore, in near future, aptamers are promising to be antibodies other biosensors replacer in detection and diagnosis assays. Various methods based on SELEX were used for selecting aptamers with high affinity and selectivity to both small and large targets independently from their toxicity and matrix effects by influencing positively the performance and accuracy of test results. This review showed main rapid, specific, robust, and highly sensitive method approaches to select aptamers for the detection of microorganisms in foods. Although the aptamer-based assays for different targets in food are still far from guarantying complete food safety, yet, the aptamer preparation systems are facilitating the development of more reliable aptamer-based commercial devices (i.e., biosensors). Thus, we emphasized frequently used combinatorial techniques in foodborne pathogen detection. Intriguingly, all methods discussed can be used for detection of other targets by substituting the aptamer. Aptamer-conjugated nanoparticles have also showed high preference given the role of nanoparticles in stabilization of aptamer and provision of additional affinity with dissociation
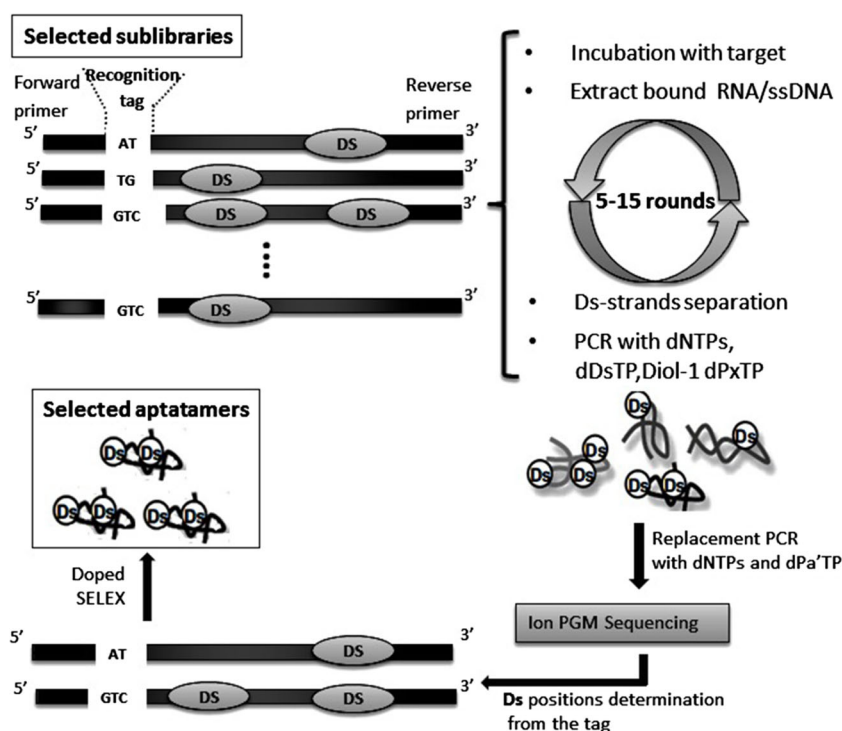
Fig. 4 Scheme of the SELEX procedure using hydrophobic unnatural base pair system for high-affinity DNA aptamer generation: The initial library is made of mixture of 22 different chemically synthesized DNA sublibraries, and each sublibrary contains 43 natural base-randomized regions with 1–3 unnatural nucleotide Ds bases at predetermined positions, flanked by constant regions of a 25-base region composed of a 5′ primer and a 2–3-natural base recognition tag on the 5′ end and a 27 base 3′ primer region. In each selection round, the single-stranded DNA library can be mixed with a target and the DNA fragments that bound to the target proteins extracted, followed by asymmetric PCR amplification involving the Ds–Px pair for the next round of selection. After seven rounds of selection, the enriched DNA library is PCR amplified in the presence of the natural dNTPs and the unnatural dPa′TP as substrates to replace the Ds bases in the DNA fragments with the natural bases (mainly A or T) via the Ds–Pa′ pair. This is followed by sequencing with Ion Torrent PGM, and then amplified DNA fragments (chemically synthesized using the natural base and Ds amidite reagents) provide the most potent aptamers towards the target(s). Finally, the selected aptamers can be optimized through doped in vitro selection to obtain the "winning" aptamers

constant ranging from nanomolar to picomolar and LOD as low as 1–10 cfu/mL. This elucidates the aptamer–nanoparticle combination ability in signal transduction which is characterized by null interference (i.e., no change in surface plasmon resonance and absorption). Furthermore, functionalization of carbon-based nanomaterials with photochromic molecules allows covalent (through cyclo-addition, condensation, or radical polymerization reaction) and non-covalent (p–p stacking, hydrophobic interaction, or electrostatic interaction) binding approach; hence, its combination with aptamer could be useful. However, discussed post-SELEX modifications frequently reduce aptamer affinity to the targets. The integration of chemically synthesized polynucleotide into the starting library (Fig. 4) and nanoparticle combination (Fig. 3) increase the aptamer stability but at an increased cost of the aptamer selection process. Hence, we propose combination of aptamer cocktails with nanomaterials for sensitivity enhancement and direct colorimetric visualization. This may prove promising for the development of aptamer-based techniques due to the shortage in detection of most foodborne parasites, some bacteria (e.g. all *Streptococcus* sp. M types except M11), viruses, and fungi towards development of feasible strips as replacer of lateral flow immunoassay for rapid detection and user friendliness. In future, leaping beyond the scope of ensuring the food safety in terms of improved detection methods, optimization of aptamer multiplex system, and whole-cell SELEX conditions may also be exploited in therapeutic arena like, but not limited to, target inhibitor aptamers or carrier aptamers of therapeutics (e.g., macular degeneration treatment) providing the insights of aptamers usage. Thus, more refinements are needed to increase aptamer and aptamer-functionalized nanoparticles and other combinatorial forms, biostability, and bioavailability to eventually tackle existing challenges.

Compliance with Ethical Standards

Conflict of Interest Omar Mukama declares that he has no conflict of interest. Jean Paul Sinumvayo declares that he has no conflict of interest. Muhammad Shamoon declares that he has no conflict of interest. Muhammad Shoaib declares that he has no conflict of interest. Henriette Mushimiyimana declares that he has no conflict of interest. Waseem Safdar declares that he has no conflict of interest. Leo Bemena declares that he has no conflict of interest. Peter Rwibasira declares that he has no conflict of interest. Samson Mugisha declares that he has no conflict of interest. Zhouping Wang declares that he has no conflict of interest.

Informed Consent Not applicable. This article does not contain any studies with human or animal subjects.

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