Aptamers: A prospective tool for infectious diseases diagnosis

Jiayi Chen | Jiahuan Zhou | Yunchi Peng | Yafeng Xie | Yongjian Xiao

INTRODUCTION

Nucleic acid aptamers are ssDNA or RNA which can specifically bind to corresponding targets with great affinity. They are obtained from a random ssDNA or RNA library synthesized in vitro through multiple rounds of screening by SELEX. In the late 1980s, many scholars attempted to isolate and screen oligonucleotides from artificially synthesized nucleotide libraries according to their biochemical properties. Eventually, in 1990, Ellington and Szostak for the first time used the SELEX technology to successfully screen out random oligonucleotides that could specifically bind to organic dyes from random RNA libraries, and these random oligonucleotides were named as “aptamers”. In the same year, Tuerk et al. successfully screened an aptamer that could specifically bind to the phage T4 DNA polymerase from the RNA library in the laboratory. Since then, technologies based on nucleic acid aptamers have been developed incessantly, giving rise to a new generation of molecular recognition probes. Because the oligonucleotide...
aptamer can fold into a three-dimensional shape by itself, it can bind to various targets non-covalently with great affinity. As molecule-level probes, aptamers can not only bind to simple molecules like ions, medicines, small molecules, and peptides, but also target intricate structures, like virus particulates, microbes, and eukaryotes. Furthermore, aptamers, also known as “chemistry antibodies”, are functionally developed as antagonists, agonists or target ligands. For instance, the anti-VEGF RNA aptamer is the first aptamer-based treatment agent, which has been accepted by the U.S. Food and Drug Administration (FDA). Currently, certain new diagnosis and treatment approaches on the basis of aptamers have been put forward and evaluated in the field of clinical application.

To date, the basic properties of aptamers have been fully delineated as follows: Aptamers are characterized by the ability to form certain three-dimensional structures, and can combine with target molecules with great specificity and affinity through the close adaptation of spatial conformations. The dissociation constant of the aptamer bound to the target molecule usually reaches the level of $10^{-9}$ to $10^{-12}$. In addition, they also have other advantages, such as wide target molecules as well as ease of synthesis and modification in vitro. And aptamers have several incomparable advantages over antibodies. First of all, aptamers are chosen via in vitro processes and don’t rely on animal models, cellular samples or even in vivo processes, whereas the production of antibodies needs to utilize living animals to activate immune response. Moreover, aptamers are easily chemically modified, which can enhance their stability, bioavailability, binding affinity and specificity. Secondly, ssDNA aptamers are stable under ambient temperatures, while antibodies need to be refrigerated to prevent denaturalization. Thirdly, in contrast to antibodies, aptamers have almost no immunogenicity and low toxicity in normal cells. Finally, aptamers can easily penetrate tissue barriers and affect cells due to their small molecules. Conversely, antibodies don’t exhibit the aforesaid advantages owing to their large molecules.

In recent years, in view of their merits, aptamers have aroused extensive academic interest and they have been utilized in the field of biomedicine, particularly in the diagnoses of cancers and infectious diseases. In the present paper, our team primarily highlight the advancements of aptamer technologies in the diagnoses of infectious diseases.

2 | SCREENING OF NUCLEIC ACID APTAMERS

Screening from random single-stranded oligonucleotide libraries to obtain highly specific nucleic acid aptamers mainly relies on SELEX, a kind of screening technology in vitro. This technique is roughly composed of four steps: binding, elution, and separation, as well as amplification (Figure 1). The traditional SELEX method is cumbersome and has a long screening period. Generally, it takes several months to screen out aptamers with high specificity and affinity to certain targets. The SELEX technology emerged for the first time in the 1990s. After the successive development for more than 30 years, the traditional SELEX technology has undergone several changes and has been incessantly combined with new experimental technologies to overcome defects and develop a series of new SELEX techniques. Overall, a lot of work has been done on the screening methods for the purpose of improving the selectivity or applicability of aptamers, shortening screening cycles and combining different binding libraries to increase the screening efficiency of target aptamers that have greatly expanded the applications of aptamers. With the expansion of screened targets, screening methods have been changed greatly and improved persistently to meet different experimental requirements. Among them, this article mainly focuses on the SELEX methods and development against various pathogens. For example, Cell-SELEX or Whole Cell-SELEX is the most commonly used technique when screening whole-cell bacteria to get aptamers that bind to living cells, including bacteria. Compared with
traditional SELEX methods, the aptamers of this method can bind the surface molecules of target cells more efficiently and should have little cross-reactivity with non-targets.21 Even a sequential toggle Cell-SELEX (STC-SELEX) has been developed on the basis of the traditional Cell-SELEX technology, and the isolated aptamers have broad reactivity to multiple bacterial targets belonging to different genera.22 When the target is a protein secreted in bacteria, such as FluMag-SELEX, the library is usually fluorescently labeled to monitor the enrichment of the library, and the target is immobilized on magnetic beads to bind the library.23 As for the target-free immobilization techniques for this class including GO-SELEX are also popular for screening small molecules, proteins and viral particles.24–26

With the fast evolution of SELEX technologies, the range of targets identified by the screened aptamers has been persistently expanded, and at the same time, the screening efficiency has been incessantly improved due to the introduction of related technologies. In conclusion, SELEX-based aptamers are bound to play increasingly pivotal roles in the field of biology.

3 | APPLICATIONS OF APATMERS

As we all know, we are under the threats of infectious diseases. With the emergence of new pathogens and the recurrence of traditional pathogens, accurate laboratory diagnosis is essential. While aptamers are highly structured single-stranded oligonucleotides whose specificity and affinity remain strong even in complex biological environments. So in the clinical diagnostic applications, researches have focused on aptamers that specifically recognize targets in various diseases including pathogen infections. It has to be admitted that the emergence of NGS and related high-throughput sequencing methods has also changed the landscape of SELEX and promoted the development of aptamers as molecular probes for bacterial diagnosis.27 In view of the characteristics of aptamers, they can exert vital effects on the diagnosis and classification of pathogenic microorganisms. Therefore, this article focuses on the application of aptamers in the detection of pathogenic microorganisms.

3.1 | Applications of aptamers in bacteria detection

At present, infectious diseases are common and frequently-occurring diseases clinically, which have threatened human health heavily. In 2019, the number of deaths caused by infectious diseases exceeded 10.2 million (95% Uncertainty Interval (UI) 6.2 million to 16.7 million), accounting for 18% of all deaths all over the world.29 Diagnosing infections mainly relies on laboratory tests, such as NGS, MassARRAY, pathogen culture and so on. Although these methods can basically meet clinical needs, they all have limitations, which are costly and cumbersome to operate. Studies have found that the characteristics of artificial mimic antibodies of aptamers can be applied to detect pathogens. In recent years, many studies on bacteria-detecting aptamers have been completed. The aptamers used for bacteria detection can be divided into two categories: one is the targeting of bacterial cell surface components, such as antigens or bacteria virulence factors; the other one is the targeting of whole cells. So far, the research on antibacterial aptamers has mainly focused on the commonly seen bacteria, such as Mycobacterium tuberculosis (M. tb), Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) and Salmonella (Table 1). (It should be noted that some of the aptamers described in this paper are based on modified nucleic acids, rather than standard RNA/DNA, and are uniformly classified into the RNA/DNA category when drawing the table.)

3.1.1 | Mycobacterium tuberculosis

Tuberculosis (TB), induced by M. tb, is one of the biggest health challenges in the world. Currently, about 1/3 of the population across the world are influenced by M. tb, and WHO speculated that approximately 10 million death cases were induced by TB in 2018.28 As for TB diagnosis, smear microscopy is a simple and rapid detection method, whereas its positive rate is merely 50%–60%,29,30 hence it is far from meeting clinical needs. Cultivating M. tb is the most accurate approach to determine the pathogene species, whereas it’s low efficient and not appropriate for point-of-care (POC) diagnoses. In addition, fast and cost-effective POC, like serologic tests, is banned for identifying TB. Tuberculin skin test (TST) and Interferon-γ-Release-Assay (IGRA) are immunologic analyses as per the principle of antigen-antibody reaction. Those test methods can’t realize the differentiation among bioactive TB, latent TB, or relapsed TB.28 Therefore, it is necessary to find more sensitive and specific methods to solve the shortcomings of current methods, and aptamers seem to be an ideal tool that meets the requirements. In 2015, Aimaiti et al. screened of aptamers targeting the standard strain of Mycobacterium tuberculosis H37Rv by the Whole Cell-SELEX. The results indicated that the combination detection of MA2/MA1 (preponderant aptamers from different groups) could effectively distinguish M. tb with high specificity and sensitivity from non-tuberculous mycobacteria or other pathogens.31 Zhang et al. chose an ssDNA aptamer against whole cells of M. tb H37Rv and established a single-walled carbon nano-tubes (SWCNT)/aptamer/Au-IDE MSPQC sensor for M. tb H37Rv identification. Especially the detection time was 70 min and the detection limit was 100cfu/ml.32 Subsequently, Mtb36, an aptamer-targeting M. tb with high binding selectivity, was developed and it combined with M. tb H37Ra strains 4 times higher than Mycobacterium bovis.33

Immediately afterwards, many scholars began to focus on the surface composition of M. tb. In 2016, Tang et al. have reported that mannose-capped lipoarabinomannan (ManLAM), as a primary constituent of the cellular wall of M. tb, is an ideal target for the early diagnosis of tuberculosis, as it is released earlier in the blood circulatory system in the process of M. tb infection. Currently, diagnosing latent TB infection (LTBI) is still a clinical challenge. Fortunately, the enzyme-linked
| Organism          | Aptamer       | Type  | Target        | Affinity (Kd) | Detection limit | Ref          |
|------------------|--------------|-------|---------------|---------------|----------------|--------------|
| M. tb H37Rv      | MA1          | DNA   | Whole-bacterium| 12.02 nM      | —              | [31]         |
| M. tb H37Rv      | Aptamer 1    | DNA   | Whole-bacterium| 37 ± 4 nM     | 100 CFU/ml     | [32]         |
| M. tb H37Ra      | Mt36         | DNA   | Whole-bacterium| 5.09±1.43 nM  | —              | [33]         |
| M. tb Beijing     | T9           | DNA   | ManLAM        | 668±59 nM     | —              | [34]         |
| M. tb H37Rv      | CE24, CE15   | DNA   | CE protein    | CE24:0.375mM  | —              | [35,36]      |
|                  |              |       |               | CE15:0.16mM   |                |              |
| M. tb             | Aptamer (17) | DNA   | MPT64         | 8.92 nM       | —              | [37]         |
| M. tb             | G43, G78     | RNA   | EsxG protein  | G43:8.04±1.90 nM | —             | [38]         |
| E. coli O157      | E-5, E-11, E-12, E-16, E-17, E-18, E-19 | DNA | LPS           | —             | —              | [41,42]      |
| E. coli O157      | AM-6         | DNA   | Whole-bacterium| 107.6 ± 67.8 nM | —              | [43]         |
| E. coli K88       | Apt B12      | DNA   | Whole-bacterium| 15 ± 4 nM     | 1.1×10^5 CFU/ml in pure culture; 2.2×10^5 CFU/g in artificially contaminated fecal sample | [44]         |
| E. coli           | P12-31       | DNA   | Whole-bacterium| —             | —              | [45]         |
| E. coli KCTC 2571 | E1, E2, E10, E12 | DNA | Whole-bacterium| E1: 12.4 nM, E2: 25.2 nM, E10: 14.2 nM, E12: 16.8 nM | 8 CFU/ml (E1) | [46,47]      |
| E. coli DH5α      | Ec3          | RNA   | Whole-cell     | 225 nM        | 2×10^4 CFU/ml  | [48]         |
| E. coli DH5α      | 8.28A        | DNA   | Whole-cell     | 27.4 ± 18.7 nM| —              | [49]         |
| S. aureus         | SA20, SA23, SA31, SA34, SA43 | DNA | Whole-bacterium| SA20:70.86±39.22 nM, SA23:61.50±22.43 nM, SA31:82.66±33.20 nM, SA34:72.42±35.23 nM, SA43:210.70±135.91 nM | 682 CFU/ml in whole blood (SA20-Conjugate-based assay) | [50]         |
| S. aureus         | SA17, SA61   | DNA   | Whole-bacterium| 34 to 128nM   | 10^2 CFU/ml (a sensitive dual labeled sandwich detection system using aptamers RAB10 and RAB35) | [51]         |
| MRSA              | PBP2a aptamer | DNA   | PBP2a         | —             | 2.63×10^3 and 1.38×10^3 CFU/ml in PBS and spiked nasal swab | [55]         |
| MRSA              | Apt-A and Apt-B | DNA | protein A and PBP2a | —             | —              | [56]         |
| S. aureus         | APTSEB1      | DNA   | SEB           | —             | —              | [160]        |
| S. aureus         | SEB          | DNA   | SEB           | 2.3×10^{-11} nM | —              | [161]        |
| S. aureus         | C1 aptamer   | DNA   | SEC1          | 65.14 ± 11.64 nM/L | 6 ng/ml SEC1 in food samples | [162]        |
| S. aureus         | R12.06       | DNA   | Alpha toxin   | 93.7±7.0 nM   | 200 nM alpha toxin in human serum samples | [163]        |
oligonucleotide assay (ELONA) of the selected aptamer T9 targeting ManLAM has certain potential merits for diagnosing LTBI such as inactive tuberculosis, smear-negative tuberculosis, extrapulmonary TB (EPTB) and immunodeficiency tuberculosis. Although it cannot distinguish between LTBI and active tuberculosis, it can assist in the diagnosis of LTBI. 34 ssDNA aptamers against ESAT6 and CFP10 (CE protein) can identify ESAT6 and CFP10 antigens in serological specimens from individuals with aPTB or EPTB or in sputum specimens. 35,36 Moreover, in 2017, Sypabekova et al., in vitro assays, screened single-stranded DNA aptamers against MPT64 protein, one of the primary excreted proteins of \textit{M. tb}. The selected aptamers exhibited good specificity and sensitivity of 90% and 91.3%, respectively, therefore, they may not easily generate false positive results and can be used for on-site tuberculosis detection and diagnosis. 37 Ngubane et al. selected 2 RNA aptamers, G43 and G78, which bound to the \textit{M. tb} EsxG protein with high affinity. Those RNA aptamers can be promising agents for diagnosing TB. 38

Owing to the persistent development of aptamers in the identification of \textit{Mycobacterium tuberculosis}, we hold that aptamers will be popularized clinically and play important roles in global tuberculosis control.

### 3.1.2 \textit{E. coli}

\textit{E. coli}, one of the most commonly seen pathogens clinically, can induce systemic multi-organ infections, such as urinary tract infections, hemorrhagic enteritis, etc. 39,40 The traditional culture-based \textit{E. coli} determination method is simple and cost-effective, whereas it often takes 2-3 days to acquire results, which is quite low efficient and fails to support on-site testing. Hence, developing rapid and sensitive \textit{E. coli} detection approaches is vital for clinical diagnoses, food safety testing, and environment monitoring. As an opportunistic pathogen, the pathogenic mechanism of \textit{E. coli} mainly depends on its own virulence factors, such as endotoxin, capsule, exotoxin, etc. Presently, many virulence strains of \textit{E. coli} have been chosen as targets for specificity aptamers, as they are ubiquitous in the gut microbiota of humans and animals, and their pathogen mutations

| Organism | Aptamer | Type | Target | Affinity (Kd) | Detection limit | Ref |
|----------|---------|------|--------|---------------|----------------|-----|
| S. aureus | Antibac1 and Antibac2 | DNA | Peptidoglycan | Antibac1: 0.415 ± 0.047 mM; Antibac2: 1.261 ± 0.280 mM | — | [164] |
| S. aureus | PA#2/8 | DNA | Protein A | 172±14 nM for the recombinant Protein A; 84±5 nM for the native Protein A | — | [165] |
| S. Typhimurium | C4 | DNA | Whole-bacterium | — | — | [60] |
| S. Typhimurium | ST2P | DNA | Whole-bacterium | 6.33 ± 0.58 nM | 25 CFU/ml | [61] |
| S. Typhimurium | SAL 26 | DNA | Whole-bacterium | 123 ± 23 nM | 10^2 CFU/ml | [62] |
| S. Enteritidis | Crn-1 and Crn-2 | DNA | Whole-bacterium | Crn-1: 0.971 μM; Crn-2: 0.309 μM | — | [63] |
| Salmonella Paratyphi A | Apt22 | DNA | Whole-bacterium | 47 ± 3 nM | 10^3 CFU/ml | [64] |
| S. Enteritidis | S25 | RNA | Mixtures of ten strains of S. Enteritidis | — | — | [58] |
| S. Typhimurium | Aptamers 33 and 45 | DNA | Outer membrane proteins | 3 CFU/ml (Aptamer 33) | — | [57,65] |
| S. Typhimurium | I-2 | RNA | OmpC protein | 20 nM | — | [66] |
| Mycoplasma | A15-1 | DNA | Whole-cell | 24.5 nM (for Jeko-1 cells) | — | [67] |
| Acinetobacter baumannii | a NC membrane-based dual aptamer | DNA | Whole-bacterium | — | 450 CFU in microfluidic system; 100 CFU in electromagnetically-driven microfluidic platform | [70,71] |
| Pseudomonas | JN08 and JN27 | DNA | Whole-bacterium | JN08: 54.9 ± 7.8 nM; JN27: 28.5 ± 4.9 nM | — | [74,75] |
| Aeruginosa | Apt | DNA | Whole-bacterium | — | 2.4 CFU/ml | [79] |
| Vibrio parahaemolyticus | A3P | DNA | Whole-bacterium | 16.88 ± 1.92 nM | 12 CFU/ml | [80,81] |
| Enterococcus faecalis | EF508 | DNA | Whole-bacterium | 37 nM | — | [83] |

\begin{table}
| Organism | Aptamer | Type | Target | Affinity (Kd) | Detection limit | Ref |
|----------|---------|------|--------|---------------|----------------|-----|
| S. aureus | Antibac1 and Antibac2 | DNA | Peptidoglycan | Antibac1: 0.415 ± 0.047 mM Antibac2: 1.261 ± 0.280 mM | — | [164] |
| S. aureus | PA#2/8 | DNA | Protein A | 172±14 nM for the recombinant Protein A; 84±5 nM for the native Protein A | — | [165] |
| S. Typhimurium | C4 | DNA | Whole-bacterium | — | — | [60] |
| S. Typhimurium | ST2P | DNA | Whole-bacterium | 6.33 ± 0.58 nM | 25 CFU/ml | [61] |
| S. Typhimurium | SAL 26 | DNA | Whole-bacterium | 123 ± 23 nM | 10^2 CFU/ml | [62] |
| S. Enteritidis | Crn-1 and Crn-2 | DNA | Whole-bacterium | Crn-1: 0.971 μM; Crn-2: 0.309 μM | — | [63] |
| Salmonella Paratyphi A | Apt22 | DNA | Whole-bacterium | 47 ± 3 nM | 10^3 CFU/ml | [64] |
| S. Enteritidis | S25 | RNA | Mixtures of ten strains of S. Enteritidis | — | — | [58] |
| S. Typhimurium | Aptamers 33 and 45 | DNA | Outer membrane proteins | 3 CFU/ml (Aptamer 33) | — | [57,65] |
| S. Typhimurium | I-2 | RNA | OmpC protein | 20 nM | — | [66] |
| Mycoplasma | A15-1 | DNA | Whole-cell | 24.5 nM (for Jeko-1 cells) | — | [67] |
| Acinetobacter baumannii | a NC membrane-based dual aptamer | DNA | Whole-bacterium | — | 450 CFU in microfluidic system; 100 CFU in electromagnetically-driven microfluidic platform | [70,71] |
| Pseudomonas | JN08 and JN27 | DNA | Whole-bacterium | JN08: 54.9 ± 7.8 nM; JN27: 28.5 ± 4.9 nM | — | [74,75] |
| Aeruginosa | Apt | DNA | Whole-bacterium | — | 2.4 CFU/ml | [79] |
| Vibrio parahaemolyticus | A3P | DNA | Whole-bacterium | 16.88 ± 1.92 nM | 12 CFU/ml | [80,81] |
| Enterococcus faecalis | EF508 | DNA | Whole-bacterium | 37 nM | — | [83] |
can elicit serious public health issues. For example, *E. coli* O157:H7 can cause multiple serious human diseases, like gastrointestinal diseases and bloody diarrhea. Fortunately, since Bruno et al. prepared single-stranded DNA aptamers for *E. coli* O157 lipopolysaccharide (LPS), those aptamers have been utilized to develop biosensors for the quick identification of *E. coli* O157:H7 in the following years. In 2017, Amraee et al. screened the single-stranded DNA aptamer AM-6 with satisfactory performance to recognize whole-cell *E. coli* O157:H7. Such specificity O157:H7 aptamer can be utilized as a diagnosis ligand and can be leveraged to identify relevant food-related illnesses.

In 2014, Peng et al. enriched a ssDNA aptamer library specific for *E. coli* K88 identification, and they proposed a sandwich identification system with different detection ranges (1.1 × 10^3 CFU/ml or 2.2 × 10^3 CFU/g) for bacteria in different samples. In 2016, Soledad used the CELL-SELEX technology and obtained an aptamer called P12-31. It's highly specific to *E. coli*. Furthermore, the aptamer can be combined with Meningitis/sepsis related *E. coli* (MNEC) clinical isolates and can bind to *E. coli* to at least 10 times stronger versus the other bacteria species studied, like *S. aureus*, *E. faecalis* and *E. freudini*. Up to now, P12-31 is the first aptamer having underlying merits for the pathological diagnoses of MNEC transmission.

In addition, there are some *E. coli* aptamers based on CELL-SELEX, which have also achieved satisfactory results. For example, Jin et al. used Aptamer E2 to develop a detection platform for quick, ultra-sensitive and specific identification of *E. coli*. Another aptamer E1 against *E. coli* KCTC 2571 was utilized for label-free and highly sensitive electrochemic identification of *E. coli* with an identification threshold of 8 CFU/ml. The method has the benefits of rapidness, low cost, simple operation, etc., and does not require an electrochemic labeling step and the supplementation of nonstable reagents. These unique advantages make it an underlying method for identifying *E. coli*-associated food borne illnesses. Dua et al. screened the RNA aptamer Ec3 targeting *E. coli* DH5α, and the identification threshold for *E. coli* cells was 2 × 10^4 CFU/ml in combination with other technologies. Renders et al. selected the aptamer 8.28A against live *E. coli* DH5α cells and discovered that the aptamer exhibited high selectivity and affinity.

All in all, due to the development of aptamer technology, as a common clinical condition pathogen and food-borne contaminating bacteria, the detection rate of *E. coli* has been greatly improved.

### 3.1.3 Staphylococcus aureus (*S. aureus*)

*S. aureus*, an anaerobic gram-positive bacterium, can cause severe systemic infections, including sepsis, enteritis and so on, due to the excretion of several exotoxins, such as enterotoxin, hemolysin, etc. Although the identification of *S. aureus* is not difficult in routine laboratories, the disease progresses rapidly once a person has been infected. These bacteria can induce lethal illnesses, especially sepsis, which seriously threatens the lives of patients. However, traditional diagnostic methods might delay the treatments for patients with severe bacterial infections, hence quick and accurate etiology diagnosis is essential. In 2009, Cao et al. first introduced a set of whole-cell specific ssDNA aptamers for the identification of *S. aureus*. Moreover, the aptamer combination can probe single *S. aureus* in pyrogenic fluids. In 2013, Chang et al. utilized the resonance light scattering signal measurement method of gold nano-particles coupled with nucleic acid aptamers, and they smoothly identified a single *S. aureus* cell in 1.5 h. Such novel technique may facilitate the development of a quick and sensitive bacteria detection method. Furthermore, in 2018, Shylaja et al. used the high-flux Whole Cell-SELEX approach to smoothly acquire specific aptamers against whole cells of *S. aureus*. The detection method they established can be a dependable approach for the routine identification of *S. aureus* in clinical specimens due to its stability and applicability.

Since *S. aureus* can induce severe outcomes, its pathogenic exotoxins cannot be ignored. Among the exotoxins, staphylococcal enterotoxin (SE) is stable, and strain-specific. The SE family consists of 23 members, ranging from SEA to SEV, of which SEA-SEI, SER, SES and SET are considered to be the primary causes of gastrointestinal diseases. Moreover, staphylococcal food poisoning (SFP) and staphylococcal enterotoxin B (SEB) mediated respiratory tract infections are commonly seen clinical symptoms. Substantial diagnosis technologies are on the basis of the serologic identification and quantified results of SEB in diverse foods and clinical specimens.

With the evolution of SELEX technologies, aptamers targeting *S. aureus* components are incessantly identified. In 2012, Jeffrey et al. screened out a new type of aptamer that had affinity for SEB in vitro, which was superior to antibodies in the identification of SE. The aptamer was named APTSEB1 and they successfully separated SEB from complex SE mixtures with remarkably high resolution. Their work has laid the foundation for the development of aptamers and the testing of the entire SE family, but needs to be ameliorated when it comes to isolation, identification, specificity magnification, and analyses. Subsequently, Mojtaba et al. obtained a set of single-stranded DNA aptamers with excellent selectivity for SEB via 12 rounds of SELEX screening in 2016. The screened aptamers were utilized to identify SEB in serological specimens. The outcomes revealed that the SEB C1 aptamer exhibited good specificity for SEB. This aptamer can be a useful tool for detecting and evaluating SEB. Furthermore, to detect SES, scientists have also developed aptamer C10 for *S. aureus* enterotoxin C1 (SEC1), aptamer R12.06 for i-toxin, aptamer Antibac1 and Antibac2 for a peptidoglycan, and aptamer PA#2/8 for protein A. Meticillin-resistant *Staphylococcus aureus* (MRSA), usually referred to as “superbug”, is a nosocomial and multi-drug resistant microbe presenting tolerance to β-lactam antimicrobials. Currently, substantial traditional approaches have been proposed to identify MRSA clinically. Cultivation-related approaches, like the disk diffusion test and microdilution approach, mainly depend on the suppressive content of antimicrobials, while DNA-derived approaches, like PCR, target the mecA gene. Those approaches have some limitations in the identification of MRSA, e.g., cultivation-related approaches are fairly low efficient. In the face of the
worldwide crisis of antibiotic resistance, there is an urgent need for new high-specificity and high-sensitivity bacterial detection strategies. Clinically, the accurate and rapid identification of MRSA is pivotal. PBP2a (penicillin binding protein 2a), an alternative to other PBPs in the crosslinking of peptidoglycan chains, is coded by the meca gene that mediates methicillin resistance. In 2018, Qiao et al. used aptamer targeting PBP2a as the basis in combination with the immunomagnetic separation technology to establish a new fluorescence analysis method for detecting MRSA in various clinical specimens. Without the time-consuming microbi culture process in conventional sensitivity tests, the total test duration of such method is merely 2 h with an identification threshold of 3.88 × 10^3 CFU/ml spiked nasal swab, respectively. This approach is easy, quick, and susceptible, and it’s suitable for the identification of MRSA. In 2020, Xu et al. proposed a new approach via integrating the dual aptamer technique and the CRISPR-Cas12a aided rolling circle amplification (RCA) method to realize the precise and ultrasensitive determination of MRSA. The functionalised aptamer can transform the protein identification into nucleic acid signal determination. Therefore, the utilization of CRISPR-Cas12a has been extended from the nucleic acid identification to microbe identification. The results show that their method has significantly improved sensitivity in the detection of MRSA. We believe that the combination of dual functionalized aptamers and CRISPR for the detection of small molecules secreted by bacteria will have more extensive applications in the field of clinical diagnosis.

To sum up, the evolution and utilization of aptamers have remarkably facilitated the early clinical diagnosis of S. aureus infection.

### 3.1.4 | Salmonella

It’s well known that *Salmonella* is an intestinal pathogen. Approximately 1.4 million patients with salmonellosis are documented every year in America, with nearly 16,000 hospitalisations and 550 mortalities. Moreover, the hospitalisations due to *Salmonella* infection accounted for 26% of all hospitalised cases of diarrhea. It is clearly a major global socioeconomic and public health burden. Among more than 2500 serovars of *Salmonella enterica*, *Salmonella enterica* subspecies *Salmonella enterica* Typhi and *Salmonella Paratyphi A* and *Salmonella Paratyphi B* can cause enteric fever that is a potentially fatal systemic illness. Some scientists have utilized Whole Cell-SELEX to determine aptamers that bind to *Salmonella Typhimurium* (S. Typhimurium), *Salmonella Paratyphi* and *Salmonella enterica serovar Enteritidis* (S. Enteritidis) for *Salmonella* identification. For example, in 2013, Yang Ming et al. used the screening technology and obtain aptamers to detect *Salmonella* paratyphi A and established a non-covalent self-assembly detection system based on single-walled carbon nano-tubes and DNAlzyme-labeled nucleic acid aptamer identification probes. The result indicated that the method could identify pathogenic bacteria in a selective and efficient manner with an identification threshold of 10^3 CFU/ml and a linearity range between 10^3 and 10^7 CFU/ml, and an identification threshold of 10^5 CFU/ml in spiked specimens. Thus, the aptamer kits available for the identification of *Salmonella enterica* in foods, clinical specimens and environment specimens are designed. Hyeon et al. utilized the mix of 10 strains of *S. Enteritidis* as targets and acquired the RNA aptamer S25, which bound to *S. Enteritidis* in a specific manner with no cross-reactivity with other *Salmonella* serovars.

In addition to using Whole Cell-SELEX screening, Joshi et al. used the S. Typhimurium outer membrane protein (OMP) as the screening target to generate aptamers 33 and 45, and Ha et al. reported a RNase-resistant RNA aptamer I-2 against S. Typhimurium OMP C.

### 3.1.5 | Applications in other bacteria

Apart from the bacteria mentioned above, aptamers are increasingly adopted in the diagnosis of other microbes. Mycoplasma is an unique cell wall-less microorganism that can induce widespread human infections, such as pneumonia, prostatitis, and non-gonococcal urethritis. Currently, the diagnosis of mycoplasma infection mainly relies on culture methods and serological tests clinically, and both methods have certain defects or deficiencies in practice. For example, mycoplasma has higher nutritional requirements for culture than general bacteria and is easy to be contaminated. Serological test results are mainly suitable for mycoplasma antibodies, which are vulnerable to the limitations of detection methods, and the results are prone to false positives and false negatives, which are difficult to eliminate. Fortunately, the application of aptamers has greatly improved the diagnosis of mycoplasma infection. In 2019, Liu et al. developed a 40-nucleotide ssDNA aptamer sequence in order to quickly detect mycoplasma-infected cells. The new aptamer probe can not only quickly detect infected cells in the laboratory, but offer an easy approach to supervise mycoplasma infections in primary cells.

*Acinetobacter baumannii* (AB) is the most common gram-negative bacillus in the genus *Acinetobacter*. This agent mainly induces respiratory tract infections, and also leads to bacteremia, urinary tract infections, secondary meningitis and so on. Furthermore, as a widely distributed pathogen in the hospital environment, AB is a great threat to critically ill patients and patients in CCU and ICU. Therefore, this type of infection is also called ICU-acquired infection. Due to its increasing infections and drug resistance, AB is drawing more and more attention from clinicians. Recently, its death rate has risen by 15%, partly due to the absence of quick and susceptible diagnosis tests. Therefore, it is of great significance to quickly and effectively monitor AB. Fortunately, the introduction of aptamers has greatly improved the diagnosis of AB infection. In 2018, Wu et al. used a nitrocellulose membrane-derived dual-aptamer test in combination with a microfluid chip for bacteria identification, and the LOD was merely 450 CFU. Due to its convenience, possibility, and susceptibility, this method has remarkably facilitated the POC diagnosis of AB. Immediately, Su et al. demonstrated an automatic POC (point-of-care) device that used a highly sensitive and specific dual aptamer test in combination with an electromagnetically driven
microfluidic system to automatically carry out AB detection within 30 min. The system is featured by fastness, sensitivity, low power consumption, low reagent consumption and complete automation, and can be used for AB double aptamer detection. It will achieve quick clinical diagnoses of AB soon enough. Moreover, the rapid detection of AB is also essential to limit health care-related infectious diseases and can offer the optimal therapy for patients.

Pseudomonas aeruginosa (P. aeruginosa) is another vital opportunistic pathogen that can induce hospital-acquired infection, featured by easy colonization, easy mutation and multi-drug resistance, and it can elicit lung infection, bloodstream infection (BSI) and bacteria sepsis. With approximately 49 million septic patients and 11 million mortalities across the globe, the early diagnosis and on-site detection of P. aeruginosa can reduce the incidence and death rate due to P. aeruginosa-induced BSI. In 2017, Jennifer used the CELL-SELEX method to obtain DNA aptamers specific for live P. aeruginosa and the result demonstrated that the aptamer exhibited a remarkable discrimination ability in the face of P. aeruginosa. Subsequently, Zhong et al. carried out the selective capture and sensitive fluorometry identification of P. aeruginosa by virtue of aptamer modified magnetism nano-particles. Such identification method could be completed in less than 1.5 h and the practicality of this method was substantiated via identifying P. aeruginosa in food specimens. In addition, Shi’s method of applying aptamers to sensors has been smoothly utilized to selectively identify P. aeruginosa in blood specimens. The biosensor is anticipated to be utilized for the quick identification of P. aeruginosa in environments, foods and clinical diagnoses, and the quick identification of P. aeruginosa is essential for accurately identifying infected individuals. Recently, the polyclonal aptamer library enriched by the FluCell-SELEX method proposed by Dennis et al. has become a novel diagnosis kit to identify carbapenem-tolerant P. aeruginosa. The remarkable convenience of the FluCell-SELEX method and the satisfactory performances of the P. aeruginosa specificity library may foster the development of novel and valid diagnosis technologies on the basis of polyclonal aptamer libraries.

Vibrio species are aerobic or facultative anaerobic fermentative gram-negative bacilli, most commonly found in water. Among them, the main pathogens to humans are Vibrio cholerae, Vibrio parahaemolyticus, which causes food poisoning caused by contaminated fish and shellfish, and Vibrio vulnificus, which can cause highly lethal sepsis. Taking Vibrio parahaemolyticus as an example, the main limitations of the current standard analytical methods for the detection of Vibrio parahaemolyticus (V.P) are cumbersome equipment, cumbersome operation and long-term. While Sadsri et al. achieved the detection of V.P through the specific recognition of Vibrio parahaemolyticus by aptamers and the formation of a special sandwich structure with a detection limit of 2.4 CFU/ml. Wu et al. introduced an aptamer-based SERS method for single-step detection of Vibrio parahaemolyticus in seafood with a detection limit of 12 CFU/ml. Enterococci, gram-positive cocci, are important pathogens for nosocomial infections, causing not only urinary tract infections, skin and soft tissue infections, but also life-threatening abdominal infections, sepsis, and meningitis. The infection is difficult to treat due to its inherent drug resistance. It is reported that 16% of nosocomial urinary tract infections are caused by Enterococcus, ranking second only to Escherichia coli. Among them, urinary tract infection is the most common infection caused by Enterococcus faecalis. The aptamer EF508 screened by Kolm et al. has a high binding affinity to E. faecalis cells with a Kd value of 37 nM and can successfully isolate E. faecalis.

3.2 Applications of aptamers in viruses

Annually, many people around the world are threatened by different kinds of viruses, such as viral hepatitis, AIDS, and new coronary pneumonia. At present, the laboratory diagnosis of viruses mainly relies on nucleic acid and serological examinations, but nucleic acid tests have the problem of missed detection, and serological examinations have defects like false negatives and insufficient sensitivity. Given the acuteness, rapid changes, and high mortality rates of viral infections, rapid and accurate diagnosis is beneficial for viral infection control. Aptamers have been developed into new detection tools that have attracted much attention, and they have shown great application potential and broad application prospects in the diagnoses and therapies of viral infections. With the evolution of new SELEX technologies, aptamers for various types of viral target molecules, such as reverse transcriptase, helicase, and viral capsid proteins, have been identified, which have greatly promoted the diagnoses for virus-infected patients. At present, the applications of aptamers in the diagnoses of virus infections mainly target viruses like hepatitis C virus (HCV), human immunodeficiency virus-1 (HIV-1), and influenza virus, etc. (Table 2).

3.2.1 HCV

HCV, a small, enveloped virus pertaining to the family Flaviviridae, has positive-sense single-stranded RNA as a genome. It is the causative agent inducing hepatitis C that might lead to hepatic fibrosis, hepatic sclerosis, and hepatic carcinoma, which makes HCV infection a main public health problem across the world. Aptamers have been utilized for HCV identification in the early phases and immuno-suppressed individuals. Chen et al. utilized DNA aptamers which can bind to HCV envelope glycoprotein E2 in a specific manner to design a HCV identification system on the basis of a sandwich-ELISA approach. Subsequently, Park et al. also used DNA aptamers against HCV E2 to make a novel aptamer-derived test system appropriate for identifying the infection titer of HCV. Lee et al. used RNA aptamers which can bind to the HCV core antigen in a specific manner to develop a chip-based detection system. Then, Pleshakova et al. designed an HCV core antigen identification system by virtue of an atom force microscope chip.
CHEN et al. (AFM-chip) with immobilised DNA aptamers. Their research revealed that the aptamers could serve as probe molecules for HCV core antigen identification in human serum at contents between $10^{-10}$ M and $10^{-13}$ M.$^{91,92}$

### 3.2.2 Influenza virus

The influenza virus, a component of the family orthomyxoviridae, is a kind of enveloped virus with a segmented negative single-stranded RNA. Amongst the 4 kinds of influenza viruses, only type A and B are clinically relevant to humans.$^{93}$ Highly infectious and variable influenza viruses have resulted in multiple severe global epidemics in the 20th century and 21st century.$^{94,95}$ Therefore, in view of its high infectivity and variability, rapid and accurate methods are urgently needed to control disease dissemination and prevent future pandemics. Fortunately, aptamers provide underlying substitute detection approaches due to their advantages.$^{96}$ The affinity of the RNA aptamer P30-10-16 is over 15 times higher compared with traditional influenza virus antibodies.$^{97}$ The majority of aptamers identified nowadays are aptamers against influenza A. Zhang et al. used an influenza A/H1N1 virus-specific aptamer to develop a fluorescence polarization platform for identifying the sequences of influenza A/H1N1 DNA.$^{98}$ Tseng et al. used a sandwich-derived anti-H1N1 virus DNA aptamer to design an automated microfluid system for the fluorescent identification of influenza A/H1N1, in which the total time consumed was approximately 0.5 h.$^{99}$ Such approach was evidently quicker in contrast to traditional virus cultivation approaches and the identification threshold was 10$^3$ times higher than the susceptibility of traditional serologic diagnoses. Wang et al. designed a microfluid system by virtue of DNA aptamers, as a single universal aptamer could induce conformation variations at diverse ionic contents, realizing the synchronous determination of 3 diverse kinds of viruses (H1N1, H3N2, and type B) in merely 20 min.$^{100}$ Lee et al. utilized an anti-H1N1 virus aptamer to make an aptamer-based

| Organism          | Aptamers                  | Type | Target          | Kd          | LOD          | Ref      |
|-------------------|---------------------------|------|-----------------|-------------|--------------|----------|
| HCV               | ZE2                       | DNA  | E2 protein      | 1.05 ± 1 nM | —            | [88]     |
| HCV               | E2-A, B, C and D          | DNA  | E2 protein      | 0.8–4 nM    | —            | [89]     |
| HCV               | 9-14 9-15                 | RNA  | Core protein    | 9-14: 142 nM; 9-15: 224 nM | —          | [90]     |
| HCV               | C4                        | DNA  | Core protein    | —           | 3.3 pg/ml    | [91]     |
| HCV               | A12, A14 and A15          | DNA  | Core protein    | —           | —            | [92]     |
| Influenza Virus   | Apt-DNA                   | DNA  | H1N1 virus      | —           | —            | [98]     |
| Influenza Virus   | —                         | DNA  | H1N1 virus      | —           | —            | [99,100] |
| Influenza Virus   | A-20                      | DNA  | H1N1 virus      | 6 nM        | —            | [102]    |
| Influenza Virus   | AP-1                      | DNA  | H1N1 virus      | —           | —            | [101]    |
| Influenza Virus   | V46                       | DNA  | H1N1 HA         | 19.2 nM     | —            | [103]    |
| Influenza Virus   | A22                       | DNA  | H3N2 HA         | 46.23 ± 5.46 nM | —         | [104,105]|
| Influenza Virus   | P30-10-16                 | RNA  | H3N2 HA         | —           | —            | [97]     |
| Influenza Virus   | RHA0385                   | DNA  | H3N2 HA         | —           | —            | [106]    |
| Influenza Virus   | Aptamer 1, 2 and 3        | DNA  | H5N1 HA         | 4.65 nM     | —            | [107-110]|
| Influenza Virus   | RHA0006                   | DNA  | H5N1 HA         | —           | —            | [111]    |
| Influenza Virus   | IF10 and IF22             | DNA  | H5N1 HA         | —           | —            | [112]    |
| HIV               | RT 26                     | DNA  | RT              | —           | —            | [115]    |
| HIV               | AntiTat5                  | RNA  | Tat             | —           | —            | [119]    |
| HBV               | anti-HBsAg RNA aptamer    | RNA  | HBsAg           | —           | —            | [120]    |
| HBV               | HO1, HO2 and HO3          | DNA  | HBsAg           | —           | —            | [121]    |
| HBV               | Aptamer 2-19              | DNA  | HBeAg           | —           | —            | [122]    |
| SARS-CoV2         | CoV2-RBD-4C               | DNA  | SARS-CoV2 spike | 19.9 nM     | —            | [126]    |
| SARS-CoV2         | RBD-PB6                   | RNA  | SARS-CoV2 spike | 18 nM       | —            | [127]    |
| SARS-CoV2         | A48                       | DNA  | SARS-CoV2 NP    | 0.49 nM     | —            | [128]    |
| SARS-CoV2         | I-C1t and I-C3t           | RNA  | SARS-CoV2 s2m   | in the nanomolar range | —         | [129]    |
| SARS-CoV2         | HAAdV-Seq4                | DNA  | SARS-CoV2 virus | 0.9 ± 0.1 nM | —          | [130]    |
| HPV               | HPV-07                    | DNA  | HPV-16 VLPs     | 400 ± 30 pM | —            | [134]    |
| HPV               | G5a3N                     | DNA  | E7              | 1.9 μM      | —            | [132]    |
Coronavirus (CoV) is a positive-sense single-stranded RNA virus pertaining to the family Coronaviridae with a genome of approximately 30 kilobases. CoV can induce different respiratory tract infections that range from mild to fatal. CoV has aroused mounting academic interest nowadays as it has induced the most serious epidemic across the world. In Dec 2019, COVID-19 was first reported in Wuhan, PRC. The virus was designated SARS-CoV2 and induces the global pandemic lasting to this day and was identified to have about 70% genetic similarity to SARS-CoV. Currently, Song et al. have discovered high-binding-affinity aptamers that target SARS-CoV-2 RBD by virtue of an ACE2 competition-derived aptamer screening method and a machine learning arithmetic. The Kd values of the optimised CoV2-RBD-1C and CoV2-RBD-4C aptamers against RBD were 5.8nM and 19.9nM, separately. And a 2'-fluorine-protected RNA aptamer against RBD screened by Valero et al. can be widely used for detection and treatment of SARS-CoV-2 and emerging variants. Zhang et al. discovered DNA aptamers against the SARS-CoV2 nucleocapsid (N) protein. Then screening of high-affinity l-DNA aptamers against SARS-CoV-2 stem-loop II-like motif (s2m) by Li et al. provides an opportunity to generate new tools and probes to study s2m function in SARS-CoV-2 and related viruses. While the DNA aptamer screened by Peinetti et al. for the whole virus increased the sensitivity to SARS-CoV-2 to 1 x 10^4 copies/ml. Those aptamers will facilitate the development of novel probes for identifying COVID-19, and they will foster the diagnoses and therapies of COVID-19 while offering novel tools for studying the causal links underneath.

Human papillomavirus (HPV) is a non-enveloped DNA virus of the family Papillomaviridae. HPV is the primary cause of cervix carcinoma and 2 strains (HPV-16, HPV-18) take up 70% of HPV-associated cervix carcinoma. Toscano-Garibay et al. separated a DNA aptamer against the HPV-16 E7 cancer protein. The separated RNA aptamer can bind to E7 in a clamp-like manner with specificity and may facilitate the identification of HPV. Leija-Montoya et al. discovered RNA aptamers against HPV-16 L1 virus-like particles (VLPs), and revealed that the separated aptamers could serve as diagnosis tools. To more validly identify HPV, Trausch et al. separated a slow off-rate modified DNA aptamer (SOMAmer) against HPV-16 VLPs. When it was utilized in ELISA, it exhibited a remarkable specificity. Their research unveiled that their method was easier and more efficient in contrast to conventional ELISA.

3.3 | Aptamer applications for special pathogens

Parasitic diseases are still serious threats to human life and health, especially in underdeveloped countries and regions, there is an urgent need to study and explore new methods of diagnosis. In recent years, research reports have screened various aptamers that can specifically recognize schistosome eggs, Leishmania in sandflies, Plasmodium in blood, and Plasmodium-infected red blood cells. They provide new avenues for the development of novel human parasite detection and control methods.
Aptamers are also used in protists. For example, trypanosoma is an important pathogenic microorganism, which can parasitize in a variety of warm-blooded and cold-blooded animals, causing great harm to the health of humans and animals. The first trypanosomal aptamer screened out is the Trypanosoma brucei aptamer. These RNA aptamers are capable of associating quickly with great affinity. In addition, they are capable of binding to other trypanosomal strains not utilized in the screening protocols; hence those aptamers may be biomarkers on the surfaces of exocellular parasites.

4 | CHALLENGES OF APPTAMER APPLICATIONS

Nucleic acid aptamers have many advantages, whereas their current development is still hindered and they are not as widely used as monoclonal antibodies. There remain certain challenges in its development. First of all, most aptamers are screened in vitro. If they enter the body as drugs, given the complex and diverse environment in the body and the shorter half-life of drugs, aptamers may not be able to exert their effects to the greatest extent. Moreover, the non-modified aptamers (particularly RNA aptamers) are usually easily digested by cell nucleases. Hence, to ameliorate the in vivo steadiness of nucleic acid aptamers, scientists often chemically modify oligonucleotides, such as modifications on the sugar ring (including F, NH2, OMe, LNA or though phosphorothioate [PS] and phosphorodithioate [PS2] linkages). Chemical modifications can not only enhance aptamers their stability, but also improve bioavailability, binding affinity, and specificity, and enable the development of therapeutic and diagnostic reagents.

Secondly, to realize the functions of aptamers in the body, a carrier is generally required. For example, when aptamers are used as drugs, scientists have to develop their corresponding carriers for delivery. The high variability of microbes and viruses and the intricate structure of the target are also extra factors which might influence the performance of the aptamer. But the systems of aptamers are considered as an effective tool for the treatment of microbial infections, with good anti-biofilm and antibacterial activities; they can reduce or inhibit the effect of bacterial toxins, and can also be used in drug delivery systems. Such as the aptamer-targeted liposome drug delivery system has been successfully customized for the treatment of Staphylococcus aureus biofilm infection.

It’s noteworthy that the large-scale commercial synthesis of aptamers is remarkably expensive. Hence, the majority of researches on aptamers were merely completed in laboratories.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

It is undeniable that aptamer-based diagnostic methods are nowadays remarkably promising in modern laboratories. Research results in biomedical fields such as bacteria and viruses show that aptamers are vital for the diagnoses and therapies of illnesses and they are essential for the evaluation of drug efficacy. In particular, the development of certain SELEX-derived technologies has gradually realized novel detection, diagnosis and treatment methods based on aptamers, and their advantages have been progressively used as alternatives or supplements to antibody reagents. In addition, therapeutic aptamers have incomparable advantages such as anti-toxin, anti-snake venom, resistance to multi-drug tolerant bacteria and virus infections, whereas their treatment efficacy and safety still needs further studies. In recent years, the first research on aptamer-specific drug tolerance in cellular cultivation has been published. Broad-spectrum aptamers may be necessary for biomedical therapies and may be pivotal for identifying their longerterm efficacy. The researches on aptamers are still not sufficient. It’s vital for researchers to persistently develop easier, more valid, and more efficient SELEX methods to discover high-specificity and/or universal aptamers against different microbes.

In addition, it’s essential to incessantly improve the detection range of aptamer visualization, and it’s vital to accelerate the application of nucleic acid aptamers in the detection, precise diagnosis, and treatment of diseases. After the comprehensive and in-depth research, we believe that aptamers can serve as remarkably valid tools pivotal for biomedical testing, diagnoses, and therapies. Normally, it's considerably time-consuming to develop a new biomedicine. Hence, it’s highly likely that we will experience constant failures, whereas we will strive steadfastly to overcome any challenge lying ahead.

Holistically, aptamers have huge application potential in the field of biomedicine. With the development of relevant researches, aptamer technologies will contribute more to the identification, diagnosis, and treatment of diseases.

FUNDING INFORMATION

The authors are funded by the General Program of the Natural Science Foundation of Hunan Province (2021JJ30609).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during this study are available from the corresponding author Yongjian Xiao on reasonable request.

ORCID

Jiayi Chen https://orcid.org/0000-0003-2953-6354

REFERENCES

1. Mutreja R, Baba SA, Navani NK. Nucleic acid aptamers as emerging tools for diagnostics and theranostics. Methods Mol Biol. 2019;2054:201-221.
2. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990;346(6287):818-822.
3. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249(4968):505-510.
48. Dua P, Ren S, Lee SW, et al. Cell-SELEX Based Identification of an RNA Aptamer for Escherichia coli and its use in various detection formats. Mol Cells. 2016;39(11):807-813.

49. Renders M, Miller E, Lam CH, et al. Whole cell-SELEX of aptamers with a tyrosine-like side chain against live bacteria. Org Biomol Chem. 2017;15(9):1980-1989.

50. Cao X, Li S, Chen L, et al. Combining use of a panel of ssDNA aptamers in the detection of Staphylococcus aureus. Nucleic Acids Res. 2009;37(14):4621-4628.

51. Chang YC, Yang CY, Sun RL, et al. Rapid single cell detection of Staphylococcus aureus by aptamer-conjugated gold nanoparticles. Sci Rep. 2013;3:1863.

52. Ramalal S, Mondal B, Lavu PS, et al. Capture and detection of Staphylococcus aureus with dual labeled aptamers to cell surface components. Int J Food Microbiol. 2018;265:74-83.

53. M AO, Yusufbeyoglu S, Ildiz N, et al. DNA aptamer-conjugated magnetic graphene oxide for pathogenic bacteria aggregation: selective and enhanced photothermal therapy for effective and rapid killing. ACS Omega. 2021;6(31):20637-20643.

54. Liu YC, Kumar K, Wu CH, et al. Selective capture and identification of methicillin-resistant Staphylococcus aureus by combining aptamer-modified magnetic nanoparticles and mass spectrometry. Int J Mol Sci. 2021;22(12):6571.

55. Qiao J, Meng X, Sun Y, et al. Aptamer-based fluorometric assay for direct identification of methicillin-resistant Staphylococcus aureus from clinical samples. J Microbiol Methods. 2018;153:92-98.

56. Xu L, Dai Q, Shi Z, et al. Accurate MRSA identification through dual-functional aptamer and CRISPR-Cas12a assisted rolling circle amplification. J Microbiol Methods. 2020;173:105917.

57. Joshi R, Janagama H, Dwivedi HP, et al. Selection, characterization, and application of DNA aptamers for the capture and detection of Salmonella enterica serovars. Mol Cell Probes. 2009;23(1):20-28.

58. Hyeon JY, Chon JW, Choi IS, et al. Development of RNA aptamers for detection of Salmonella Enteritidis. J Microbiol Methods. 2012;89(1):79-82.

59. Awang MS, Bustami Y, Hamzah HH, et al. Advancement in salmonella detection methods: from conventional to electrochemical-based sensing detection. Biosensors (Basel). 2021;11(9):346.

60. Moon J, Kim G, Lee S, et al. Identification of salmonella typhimurium-specific DNA aptamers developed using whole-cell SELEX and FACS analysis. J Microbiol Methods. 2013;95(2):162-166.

61. Duan N, Wu S, Chen X, et al. Selection and characterization of aptamers against Salmonella typhimurium using whole-bacterium systemic evolution of ligands by exponential enrichment (SELEX). J Agric Food Chem. 2013;61(13):3229-3234.

62. Lavu PS, Mondal B, Ramalal S, et al. Selection and characterization of aptamers using a modified whole cell bacterium SELEX for the Detection of Salmonella enterica serovar typhimurium. ACS Comb Sci. 2016;18(6):292-301.

63. Bayrçç Ç, Eyidoğan F, Avni Öktem H. DNA aptamer-based colorimetric detection platform for Salmonella Enteritidis. Biosens Bioelectron. 2017;98:22-28.

64. Yang M, Peng Z, Ning Y, et al. Highly specific and cost-effective detection of Salmonella Paratyphi A combining aptamers with single-walled carbon nanotubes. Sensors (Basel). 2013;13(5):6865-6881.

65. Ma X, Jiang Y, Jia F, et al. An aptamer-based electrochemical biosensor for the detection of Salmonella. J Microbiol Methods. 2014;98:94-98.

66. Han SR, Lee SW. In vitro selection of RNA aptamer specific to Salmonella typhimurium. J Microbiol Biotechnol. 2013;23(6):878-884.

67. Liu Y, Jiang W, Yang S, et al. Rapid detection of mycoplasma-infected cells by an ssDNA aptamer probe. ACS Sens. 2019;4(8):2028-2038.

68. Strom M, Crowley T, Shigdar S. Novel detection of nasty bugs, prevention is better than cure. Int J Mol Sci. 2020;22(1):149.

69. Rasoulinejad S, Gargari SLM. Aptamer-nanobody based ELASA for specific detection of Acinetobacter baumannii isolates. J Biotechnol. 2016;231:46-54.

70. Wu JH, Wang CH, Ma YD, et al. A nitrocellulose membrane-based integrated microfluidic system for bacterial detection utilizing magnetic-composite membrane microdevices and bacteria-specific aptamers. Lab Chip. 2018;18(11):1633-1640.

71. Su CH, Tsai MH, Lin CY, et al. Dual aptamer assay for detection of Acinetobacter baumannii on an electromagnetically-driven microfluidic platform. Biosens Bioelectron. 2020;159:121148.

72. Zheng X, Gao S, Wu J, et al. Recent advances in aptamer-based biosensors for detection of Pseudomonas aeruginosa. Frontiers in Microbiology. 2020;11:605229.

73. Hart RJ, Morici LA. Vaccination to prevent Pseudomonas aeruginosa bloodstream infections. Front Microbiol. 2022;13:870104.

74. Soundy J, Day D. Selection of DNA aptamers specific for live Pseudomonas aeruginosa. PLoS ONE. 2017;12(9):e0185385.

75. Zhong Z, Gao X, Gao R, et al. Selective capture and sensitive fluorometric determination of Pseudomonas aeruginosa by using aptamer modified magnetic nanoparticles. Micrornach Acta. 2018;185(8):377.

76. Shi X, Zhang J, He F. A new aptamer/polyadenylated DNA interdigitated gold electrode piezoelectric sensor for rapid detection of Pseudomonas aeruginosa. Biosens Bioelectron. 2019;132:224-229.

77. Kubiecz D, Raber H, Bodenberger N, et al. The diversity of a polyclonal FluCell-SELEX library outperforms individual aptamers as emerging diagnostic tools for the identification of carbapenem resistant Pseudomonas aeruginosa. Chemistry. 2020;26(4):14536-14545.

78. Wang W, Tan L, Wu J, et al. A universal signal-on electrochemical assay for rapid on-site quantitation of vibrio parahaemolyticus using aptamer modified magnetic metal-organic framework and phenylboronic acid-ferrocene co-immobilized nanolabel. Anal Chim Acta. 2020;1133:128-136.

79. Sadsri V, Trakulsujaritthok T, Tangwattanachueeporn M, et al. Simple colorimetric assay for Vibrio parahaemolyticus detection using aptamer-functionalized nanoparticles. ACS Omega. 2020;5(34):21437-21442.

80. Wu S, Duan N, Shen M, et al. Surface-enhanced Raman spectroscopic single step detection of Vibrio parahaemolyticus using gold coated polydimethylsiloxane as the active substrate and aptamer modified gold nanoparticles. Mikrochim Acta. 2019;186(7):401.

81. Duan N, Wu S, Chen X, et al. Selection and identification of a DNA aptamer targeted to Vibrio parahaemolyticus. J Agric Food Chem. 2012;60(16):4034-4038.

82. Malik RK, Montecalvo MA. Epidemiology and control of vancomycin-resistant enterococci in a regional neonatal intensive care unit. Pediatr Infect Dis J. 1999;18(4):352-356.

83. Kolm C, Cervenka I, Aschl UJ, et al. DNA aptamers against bacterial cells can be efficiently selected by a SELEX process using state-of-the-art qPCR and ultra-deep sequencing. Sci Rep. 2020;10(1):20917.

84. Dunning J, Ballie J, Cao B, et al. Antiviral combinations for severe influenza. Lancet Infect Dis. 2014;14(12):1259-1270.

85. Sahu GK. Potential implication of residual viremia in patients on effective antiretroviral therapy. AIDS Res Hum Retroviruses. 2015;31(1):25-35.

86. Marascio N, Torti C, Liberto M, et al. Update on different aspects of HCV variability: focus on NS5B polymerase. BMC Infect Dis. 2014;14 Suppl 5(Suppl 5):S1.
Pleshakova TO, Kaysheva AL, Shumov ID, et al. Detection of hepatitis C virus core antigen using afm chips with immobilized aptamers. J Virol Methods. 2018;251:99-105.

Pleshakova TO, Kayshova AL, Shumov ID, et al. Detection of hepatitis C virus core protein in serum using aptamer-functionalized AFM chips. Micromachines (Basel). 2019;10(2):129.

Arbeitskreis Blut U. Influenza virus. Transfus Med Hemother. 2009;36(1):32-39.

Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature. 2009;459(7249):931-939.

Tharakaraman K, Sasisekharan R. Influenza surveillance: 2014-2015 H1N1 “swine”-derived influenza viruses from India. Cell Host Microbe. 2015;17(3):279-282.

Zavyalova E, Kopylov A. Aptamers to hemagglutinin: a novel tool for influenza virus recognition and neutralization. Curr Pharm Des. 2016;22(31):4835-4853.

Misono TS, Kumar PK. Selection of RNA aptamers against human influenza virus hemagglutinin using surface plasmon resonance. Anal Biochem. 2005;342(2):312-317.

Zhang J, Tian J, He Y, et al. Protein-binding aptamer assisted signal amplification for the detection of influenza A (H1N1) DNA sequences based on quantum dot fluorescence polarization analysis. Analyst. 2013;138(17):4722-4727.

Tseng YT, Wang CH, Chang CP, et al. Integrated microfluidic system for rapid detection of influenza H1N1 virus using a sandwich-based aptamer assay. Biosens Bioelectron. 2016;82:105-111.

Wang CH, Chang CP, Lee GB. Integrated microfluidic device using a single universal aptamer to detect multiple types of influenza viruses. Biosens Bioelectron. 2016;86:247-254.

Lee JM, Kim J, Ryu I, et al. An aptamer-based electrochemical sensor that can distinguish influenza virus subtype H1 from H5. J Microbiol Biotechnol. 2017;27(11):2037-2043.

Bai C, Lu Z, Jiang H, et al. Aptamer selection and application in multivalent binding-based electrical impedance detection of inactivated H1N1 virus. Biosens Bioelectron. 2018;110:162-167.

Bhardwaj J, Chaudhary N, Kim H, et al. Subtyping of influenza A H1N1 virus using a label-free electrochemical biosensor based on the DNA aptamer targeting the stem region of HA protein. Anal Chim Acta. 2019;1064:94-103.

Chen C, Zou Z, Chen L, et al. Functionalized magnetic microparticle-based colorimetric platform for influenza A virus detection. Nanotechnology. 2016;27(43):435102.

Cui ZQ, Ren Q, Wei HP, et al. Quantum dot-aptamer nanoprobes for recognizing and labeling influenza A virus particles. Nanoscale. 2011;3(6):2454-2457.

Kukushkin VI, Ivanov NM, Novoseltseva AA, et al. Highly sensitive detection of influenza virus with SERS aptasensor. PLoS ONE. 2019;14(4):e0216247.

Bai H, Wang R, Hargis B, et al. ASPR aptasensor for detection of avian influenza virus H5N1. Sensors (Basel). 2012;12(9):12506-12518.

Wang R, Li Y. Hydrogel based QCM aptasensor for detection of avian influenza virus. Biosens Bioelectron. 2013;42:148-155.

Lum J, Wang R, Hargis B, et al. An impedance aptasensor with microfluidic chips for specific detection of H5N1 avian influenza virus. Sensors (Basel). 2015;15(8):18565-18578.

Karash S, Wang R, Kelso L, et al. Rapid detection of avian influenza virus H5N1 in chicken tracheal samples using an impedance aptasensor with gold nanoparticles for signal amplification. J Virol Methods. 2016;236:147-156.

Pang Y, Rong Z, Wang J, et al. A fluorescent aptasensor for H5N1 influenza virus detection based-on the core-shell nanoparticles metal-enhanced fluorescence (MEF). Biosens Bioelectron. 2015;66:527-532.

Nguyen VT, Seo HB, Kim BC, et al. Highly sensitive sandwich-type SPR based detection of whole H5N1 viruses using a pair of aptamers. Biosens Bioelectron. 2016;86:293-300.

Weiss RA. How does HIV cause AIDS? Science. 1993;260(5112):1273-1279.

Chan DC, Kim PS. HIV entry and its inhibition. Cell. 1998;99(5):681-684.

Pavski V, Le XC. Detection of human immunodeficiency virus type 1 reverse transcriptase using aptamers as probes in affinity capillary electrophoresis. Anal Chem. 2001;73(24):6070-6076.

Yamamoto R, Baba T, Kumar PK. Molecular beacon aptamer fluoroses in the presence of Tat protein of HIV-1. Genes Cells. 2000;5(5):389-396.

Minunni M, Tombelli S, Gullotto A, et al. Development of biosensors with aptamers as bio-recognition element: the case of HIV-1 Tat protein. Biosens Bioelectron. 2004;20(6):1149-1156.

Tombelli S, Minunni M, Luzi E, et al. Aptamer-based biosensors for the detection of HIV-1 Tat protein. Bioelectrochemistry. 2005;67(2):135-141.

Caglayan MO, Üstündag Z. Spectrophotometric ellipsometry based Tat-protein RNA-aptasensor for HIV-1 diagnosis. Spectrochim Acta A Mol Biomol Spectrosc. 2020;227:117748.

Suh SK, Song S, Oh HB, et al. Aptamer-based competitive binding assay for one-step quantitation of hepatitis B surface antigen. Analyst. 2014;139(17):4310-4314.

Xi Z, Huang R, Li Z, et al. Selection of HBsAg-specific DNA aptamers based on carboxylated magnetic nanoparticles and their application in the rapid and simple detection of hepatitis B virus infection. ACS Appl Mater Interfaces. 2015;7(21):11215-11223.

Huang R, Xi Z, Deng Y, et al. Fluorescence based aptasensors for the determination of hepatitis B virus e antigen. Sci Rep. 2016:6:31103.

Fehr AR, Perlman S. Coronavirusns: an overview of their replication and pathogenesis. Methods Mol Biol. 2015;1282:1-23.

Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005;309(5742):1864-1868.

Hui DS, Azhar E, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - the latest 2019 novel coronavirus outbreak in Wuhan. China. Int J Infect Dis. 2020;91:264-266.

Song Y, Song J, Wei X, et al. Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein. Anal Chem. 2020;92(14):9895-9900.

Valero J, Civit L, Dupont DM, et al. A serum-stable RNA aptamer specific for SARS-CoV-2 neutralizes viral entry. Proc Natl Acad Sci USA. 2021;118(50):e2112942118.

Zhang L, Fang X, Liu X, et al. Discovery of sandwich-type COVID-19 nucleocapsid protein DNA aptamers. Chem Commun (Camb). 2020;56(70):10235-10238.

Li J, Sczepanski JT. Targeting a conserved structural element from the SARS-CoV-2 genome using I-DNA aptamers. RSC Chem Biol. 2022;3(1):79-84.

Peinetti AS, Lake RJ, Cong W, et al. Direct detection of human adenovirus or SARS-CoV-2 with ability to inform infectivity using DNA aptamer-nanopore sensors. Sci Adv. 2021;7(39):eabg2848.

Ljubojevic S, Skerlev M. HPV-associated diseases. Clin Dermatol. 2014;32(2):227-234.

Toscano-Garibay JD, Benitez-Hess ML, Alvarez-Salas LM. Isolation and characterization of an RNA aptamer for the HPV-16 E7 oncoprotein. Arch Med Res. 2011;42(2):88-96.
133. Leija-Montoya AG, Benitez-Hess ML, Toscano-Garibay JD, et al. Characterization of an RNA aptamer against HPV-16 L1 virus-like particles. *Nucleic Acids Res*. 2014;24(5):344-355.

134. Trausch JJ, Shank-Retzlaff M, Verch T. Development and characterization of an HPV type-16 specific modified DNA aptamer for the Improvement of potency assays. *Anal Chem*. 2017;89(6):3554-3561.

135. Long Y, Qin Z, Duan M, et al. Screening and identification of DNA aptamers toward *Schistosoma japonicum* eggs via SELEX. *Sci Rep*. 2016;6:24986.

136. Niederlender S, Fontaine JJ, Karadjian G. Potential applications of aptamers in veterinary science. *Vet Res*. 2021;52(1):79.

137. Bruno JG, Richarte AM, Phillips T, et al. Development of a fluorescent enzyme-linked DNA aptamer-magnetic bead sandwich assay and portable fluorometer for sensitive and rapid leishmaniasis detection in sandflies. *J Fluoresc*. 2014;24(1):267-277.

138. Xu Y, Jiang X, Zhou Y, et al. Systematic evolution of ligands by exponential enrichment technologies and aptamer-based applications: recent progress and challenges in precision medicine of infectious diseases. *Front Bioeng Biotechnol*. 2021;9:704077.

139. Lee S, Manjunatha DH, Jeon W, et al. Cationic surfactant-based colorimetric detection of plasmodium lactate dehydrogenase, a biomarker for malaria, using the specific DNA aptamer. *PLoS One*. 2014;9(7):e100847.

140. Cheung YW, Dirkzwager RM, Han J, et al. Identification of malaria parasite-infected red blood cell surface aptamers by inertial microfluidic SELEX (I-SELEX). *Sci Rep*. 2018;14(15):131-136.

141. Lee J, Kim TI, Ke HG, et al. Genetic diversity of *Plasmodium vivax* and *Plasmodium falciparum* lactate dehydrogenases in Myanmar isolates. *Malar J*. 2020;19(1):60.

142. Birch CM, Hou HW, Han J, et al. Identification of malaria parasite-infected red blood cell surface aptamers by inertial microfluidic SELEX (I-SELEX). *Sci Rep*. 2015;5:11347.

143. Oteng EK, Gu W, McKeague M. High-efficiency enrichment enables identification of aptamers to circulating *Plasmodium falciparum*-infected erythrocytes. *Sci Rep*. 2020;10(1):9706.

144. Nagarkatti R, de Araújo FF, Gupta C, et al. Aptamer-based, non-PCR, non-serological detection of Chagas disease biomarkers in *Trypanosoma cruzi* infected mice. *PLoS Negl Trop Dis*. 2014;8(1):e2650.

145. Ospina-Villa JD, Cisneros-Sarabia A, Sanchez-Jimenez MM, et al. Current advances in the development of diagnostic tests based on aptamers in parasitology: a systematic review. *Pharmaceutics*. 2020;12(11):1046.

146. Homann M, Göringer HU. Combinatorial selection of high affinity RNA ligands to live African trypanosomes. *Nucleic Acids Res*. 1999;27(9):2006-2014.

147. Thiel KW, Giangrande PH. Therapeutic applications of DNA and RNA aptamers. *Oligonucleotides*. 2009;19(3):209-222.

148. Chan KY, Kinghorn AB, Hollenstein M, et al. Chemical modifications for a next generation of nucleic acid aptamers. *ChemBiochem*. 2022;23(15):e202200006.

149. McKenzie LK, El-Khoury R, Thorpe JD et al. Recent progress in non-native nucleic acid modifications. *Chem Soc Rev*. 2021;50(8):5126-5164.

150. Ni S, Zhuo Z, Pan Y, et al. Recent progress in aptamer discovery and modifications for therapeutic applications. *ACS Appl Mater Interfaces*. 2021;13(8):9500-9519.

151. Ren X, Gelinas AD, Linehan M, et al. Evolving a RIG-I antagonist: a modified DNA aptamer mimics viral RNA. *J Mol Biol*. 2021;433(21):167227.

152. Pan Q, Luo F, Liu M, et al. Oligonucleotide aptamers: promising and powerful diagnostic and therapeutic tools for infectious diseases. *J Infect*. 2018;77(2):83-98.

153. Afrasiabi S, Pourhajibagher M, Raoofian R, et al. Therapeutic applications of nucleic acid aptamers in microbial infections. *J Biomed Sci*. 2020;27(1):6.

154. Wan Q, Liu X, Zu Y. Oligonucleotide aptamers for pathogen detection and infectious disease control. *Theranostics*. 2021;11(18):9133-9161.

155. Ommen P, Hansen L, Hansen BK, et al. Aptamer-targeted drug delivery for *Staphylococcus aureus* biofilm. *Front Cell Infect Microbiol*. 2022;12:814340.

156. Zhou J, Rossi J. Aptamers as targeted therapeutics: current potential and challenges. *Nat Rev Drug Discov*. 2017;16(3):181-202.

157. Lange MJ, Nguyen PDM, Callaway MK, et al. RNA-protein interactions govern antiviral specificity and encapsidation of broad spectrum anti-HIV reverse transcriptase aptamers. *Nucleic Acids Res*. 2017;45(10):6087-6097.

158. González VM, et al. Use of aptamers as diagnostics tools and antiviral agents for human viruses. *Pharmaceuticals (Basel)*. 2016;9(4):78.

159. Ospina-Villa JD, Zamorano-Carrillo A, Castanon-Sanchez CA, et al. Aptamers as a promising approach for the control of parasitic diseases. *Braz J Infect Dis*. 2016;20(6):610-618.

160. DeGrasse JA. A single-stranded DNA aptamer that selectively binds to *Staphylococcus aureus* enterotoxin B. *PLoS ONE*. 2012;7(3):e33410.

161. Hedayati Ch M, Amani J, Sedighian H, et al. Isolation of a new ssDNA aptamer against staphylococcal enterotoxin B based on CNBr-activated sepharose-4B affinity chromatography. *J Mol Recognit*. 2016;29(9):436-445.

162. Huang Y, Chen X, Duan N, et al. Selection and characterization of DNA aptamers against *Staphylococcus aureus* enterotoxin C1. *Food Chem*. 2015;166:623-629.

163. Hong KL, Battistella L, Salva AD, et al. In vitro selection of single-stranded DNA molecular recognition elements against *S. aureus* alpha toxin and sensitive detection in human serum. *Int J Mol Sci*. 2015;16(2):2794-2809.

164. Ferreira IM, de Souza Lacerda CM, de Faria LS, et al. Selection of peptidoglycan-specific aptamers for bacterial cells identification. *Appl Biochem Biotechnol*. 2014;174(1):2548-2556.

165. Stoltenburg R, Schubert T, Strehlitz B. In vitro selection and interaction studies of a DNA aptamer targeting protein A. *PLoS ONE*. 2015;10(7):e0134403.

**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Chen J, Zhou J, Peng Y, Xie Y, Xiao Y. Aptamers: A prospective tool for infectious diseases diagnosis. *J Clin Lab Anal*. 2022;36:e24725. doi: 10.1002/jcla.24725