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REVIEW ARTICLE

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Aptamers: A prospective tool for infectious diseases diagnosis

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Abstract

It is well known that people's health is seriously threatened by various pathogens (such as *Mycobacterium tuberculosis*, *Treponema pallidum*, Novel coronavirus, HIV, Mucor, etc.), which leads to heavy socioeconomic burdens. Therefore, early and accurate pathogen diagnosis is essential for timely and effective therapies. Up to now, diagnosing human contagious diseases at molecule and nano levels is remarkably difficult owing to insufficient valid probes when it comes to determining the biological markers of pathogens. Aptamers are a set of high-specificity and high-sensitivity plastic oligonucleotides screened in vitro via the selective expansion of ligands by exponential enrichment (SELEX). With the advent of aptamer-based technologies, their merits have aroused mounting academic interest. In recent years, as new detection and treatment tools, nucleic acid aptamers have been extensively utilized in the field of biomedicine, such as pathogen detection, new drug development, clinical diagnosis, nanotechnology, etc. However, the traditional SELEX method is cumbersome and has a long screening cycle, and it takes several months to screen out aptamers with high specificity. With the persistent development of SELEX-based aptamer screening technologies, the application scenarios of aptamers have become more and more extensive. The present research briefly reviews the research progress of nucleic acid aptamers in the field of biomedicine, especially in the diagnosis of contagious diseases.

KEYWORDS
aptamer, diagnosis, infectious disease, selective expansion of ligands by exponential enrichment, ssDNA

1 | 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.[1] 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".[2] 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.[3] 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.[4] As molecule-level probes, aptamers can not only bind to simple molecules like ions, medicines, small molecules, and peptides, but 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.[5,6]

To date, the basic properties of aptamers have been fully delineated as follows.[7–11] 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}~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.[12–20]

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

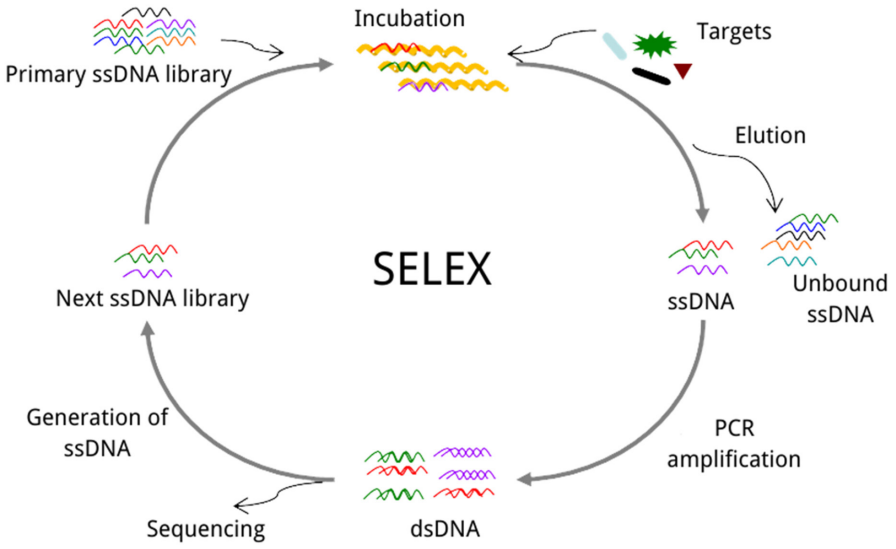

FIGURE 1 The schematic flow for the SELEX process

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 APTAMERS

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

TABLE 1 Summary of bacteria aptamers for diagnosis

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	Mtb36	DNA	Whole-bacterium	5.09±1.43 nM	—	[33]
M. tb Beijing strains	T9	DNA	ManLAM	668±59 nM	—	[34]
M. tb H37Rv	CE24, CE15	DNA	CE protein	CE24:0.375mM CE15:0.16mM	—	[35,36]
M. tb	Aptamer (17)	DNA	MPT64	8.92 nM	—	[37]
M. tb	G43, G78	RNA	EsxG protein	G43:8.04±1.90 nM G78:78.85±9.40 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 pmol	—	[43]
E. coli K88	Apt B12	DNA	Whole-bacterium	15 ±4 nM	1.1×10^3 CFU/ml in pure culture; 2.2×10^3 CFU/g in artificially contamina ted 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	2×10^4CFU/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 SA31:82.86 ±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	SA17: 35nM SA61: 129nM	Single cell	[51]
S. aureus	RAB10, RAB20, RAB28, RAB35	DNA	Whole-bacterium	34 to 128nM	10^2 CFU/ml (a sensitive dual labeled sandwich detection system using aptamers RAB10 and RAB 35	[52]
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	APT^{SEB1}	DNA	SEB	—	—	[160]
S. aureus	SEB C1 aptamer	DNA	SEB	2.3×10^{-11}nM	—	[161]
S. aureus	C10	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]

TABLE 1 (Continued)

Organism	Aptamer	Type	Target	Affinity (Kd)	Detection limit	Ref
S. aureus	Antibac1and Antibac2	DNA	Peptidoglycan	Antibac1: 0.415 ±0.047mM Antibac2: 1.261 ±0.280mM	—	[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]
Pseudomoas	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]

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 *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 *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 *Mycobacterium tuberculosis*, we hold that aptamers will be popularized clinically and play important roles in global tuberculosis control.

3.1.2 | *E. coli*

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 *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 *E. coli* detection approaches is vital for clinical diagnoses, food safety testing, and environment monitoring. As an opportunistic pathogen, the pathogenic mechanism of *E. coli* mainly depends on its own virulence factors, such as endotoxin, capsule, exotoxin, etc. Presently, many virulence strains of *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

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.[41,42] In 2017, Amraee et al. screened the single-stranded DNA aptamer AM-6 with satisfactory performance to recognize whole-cell *E. coli* O157:H7.[43] 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.[44] 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* 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.[45]

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*.[46] 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.[47] 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.[48] Renders et al. selected the aptamer 8.28A against live *E. coli* DH5α cells and discovered that the aptamer exhibited high selectivity and affinity.[49]

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*.[50] Moreover, the aptamer combination can probe single *S. aureus* in pyogenic 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.[51] 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*.[52] 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 SEIV, of which SEA-SEI, SER, SES and SET are considered to be the primary causes of gastrointestinal diseases.[51] 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.[52] 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.[53] 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.[52] 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 □-toxin, aptamer Antibac1 and Antibac2 for a peptidoglycan, and aptamer PA#2/8 for protein A.[54–57]

Methicillin-resistant *Staphylococcus aureus* (MRSA), usually referred to as "superbug", is a nosocomial and multi-drug resistant microbe presenting tolerance to β-lactam antimicrobials.[53] 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.[54] 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 microbe culture process in conventional sensitivity tests, the total test duration of such method is merely 2 h with an identification threshold of 1.38×10^3 CFU/ml spiked nasal swab, respectively. This approach is easy, quick, and susceptible, and it's suitable for the identification of MRSA.[55] 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.[56] 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.[57] It is clearly a major global socioeconomic and public health burden.[58] Among more than 2500 serovars of *Salmonella enterica*, *Salmonella enterica* subspecies serovars Typhi and Paratyphi A, B and C can cause enteric fever that is a potentially fatal systemic illness.[59] 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.[60–63] 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 DNAzyme-labeled nucleic acid aptamer identification probes.[64] 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 10^4 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.[58]

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.[57,65,66]

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.[67] 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.[68,69] Therefore, it is of great significance to quickly and effectively monitor AB. Fortunately, the introduction of aptamers has been 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.[70] 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.[71] 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.[72,73] 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*.[74] Subsequently, Zhong et al. carried out the selective capture and sensitive fluorometry identification of *P. aeruginosa* by virtue of aptamer modified magnetism nano-particles.[75] 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.[76] 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*.[77] 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 longtime.[78] 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.[79] 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.[80,81]

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*.[82] 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*.[83]

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.[84–86] 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).[87]

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 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.[88] 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.[89] 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.[90] Then, Pleshakova et al. designed an HCV core antigen identification system by virtue of an atom force microscope chip

TABLE 2 Summary of viruses aptamers for diagnosis

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	—	3.3pg/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-I	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	l-C1t and l-C3t	RNA	SARS-CoV2 s2m	in the nanomolar range	—	[129]
SARS-CoV2	HAdV-Seq4	DNA	SARS-CoV2 virus	0.9 ± 0.1 nM	10^4copies/ml	[130]
HPV	HPV-07	DNA	HPV-16 VLPs	400 ± 30 pM	—	[134]
HPV	G5a3N	DNA	E7	1.9 μM	—	[132]

(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

electrochemical sensor which was capable of distinguishing the H1 sub-type from the H5 sub-type as a molecule-level probe.[101] Bai et al. discovered a DNA aptamer via SELEX and the aptamer could identify a target at contents of merely 0.3 ng/μL by enzyme-linked oligonucleotide assay (ELONA).[102] Bhardwaj et al. used a DNA aptamer specific for the H1N1 virus HA stem region to identify the targeted virus at contents of 3.7pfu/ml.[103]

Apart from those aforesaid aptamers, there are aptamers for H3N2 virus, such as A22,[104,105] RHA0385,[106] etc., and there are aptamers for H5N1 virus, such as Aptamer 1, 2 and 3,[107–110] RHA0006,[111] IF10 and IF22.[112]

3.2.3 | HIV

Human immunodeficiency virus, a lentivirus (a sub-group of retroviruses), induces acquired immunodeficiency syndrome (AIDS).[113] When the quantity of $CD4^+$ T cells reduce to a critical content, cell-mediated immune activity is lost, and the body becomes more vulnerable to various infectious diseases.[114] Considering the increasing infection rate of HIV, accurate and rapid diagnosis is imperative for its prevention and treatment. Using aptamers may be an excellent alternative to facilitate HIV diagnosis. Numerous scholars utilize aptamers to diagnose HIV. Pavski et al. utilized a specificity DNA aptamer for HIV-1's reverse transcriptase (RT) as a probe to develop an affinity capillary electrophoresis/laser-induced fluorescent (CE/LIF) test method.[115] Moreover, Yamamoto et al. screened RNA aptamers against the Tat protein of HIV-1.[116] Their research reported that RNA aptamers specific for Tat exhibited greater binding affinity in contrast to its canonical TAR RNA binding partner on HIV-1 RNA. Mascini's lab used a specificity RNA aptamer for the HIV-1 Tat protein to design 2 diverse types of aptamer-derived sensors.[117,118] Caglayan et al. used a specific RNA aptamer to develop a RNA-aptasensor and their results displayed great HIV-Tat protein identification rates.[119]

3.2.4 | Other viruses

Hepatitis B virus (HBV) is a partly double-stranded DNA virus pertaining to the Hepadnaviridae family and is categorized into 8 gene types ranging from A to H. HBV can induce hepatitis B, which can be an acute or chronic disease eliciting hepatic sclerosis and liver cell cancer.[87] To validly identify HBV, Suh et al. used specific RNA aptamers to develop an identification device for the HBV surface antigen (HBsAg).[120] Their research revealed that their approach was nearly 40 times more valid in contrast to the most extensively utilized traditional identification methods. Xi et al. designed HBsAg-specificity DNA aptamers attached to carboxylated magnetism nano-particles (MNPs).[121] Such method's identification threshold was 5 times better in contrast to traditional ELISA. Huang et al. used a specific DNA aptamer to identify HBV e antigen (HBeAg) and the aptamer could be the molecule-leve identification element and validly identify HBeAg in blood serum in 120 s.[122]

Coronavirus (CoV) is a positive-sense single-stranded RNA virus pertaining to the family Coronaviridae with a genome of approximately 30 kilobases.[123] 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.[124] 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.[125] 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 5.8 nM and 19.9 nM, separately.[126] 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.[127] Zhang et al. discovered DNA aptamers against the SARS-CoV2 nucleocapsid (N) protein.[128] 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.[129] While the DNA aptamer screened by Peinetti et al. for the whole virus increased the sensitivity to SARS-CoV-2 to 1×10^4 copies/ml.[130] 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 Papilomaviridae. HPV is the primary cause of cervix carcinoma and 2 strains (HPV-16, HPV-18) take up 70% of HPV-associated cervix carcinoma.[131] Toscano-Garibay et al. separated a RNA aptamer against the HPV-16 E7 cancer protein.[132] 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.[133] 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.[134]

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,[135,136] Leishmania in sandflies,[137,138] Plasmodium in blood,[139–141] and Plasmodium-infected red blood cells.[142,143] They provide new avenues for the development of novel human parasite detection and control methods.

Aptamers are also used in protists.[144,145] 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.[146] 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 APTAMER 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.[147] 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 phosphorodiothioate [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.[148–151]

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.[152] 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.[153,154] Such as the aptamer-targeted liposome drug delivery system has been successfully customized for the treatment of *Staphylococcus aureus* biofilm infection.[155]

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.[156] 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.[157] Broad-spectrum aptamers may be necessary for biomedical therapies and may be pivotal for identifying their longterm 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.[158,159] 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.

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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.

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SUPPORTING INFORMATION

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

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