Feng *et al. Virology Journal* (2024) 21:237
https://doi.org/10.1186/s12985-024-02513-9

Virology Journal

REVIEW Open Access

Advances in nucleic acid aptamer-based detection of respiratory virus and bacteria: a mini review

Rui-Min Feng[1,2,5], Ye Liu[1,2], Zhi-Qiang Liu[3], Li Wang[1], Nan Chen[2], Yu Zhao[4*] and Hua-Wei Yi[1,3*]

Abstract

Respiratory pathogens infecting the human respiratory system are characterized by their diversity, high infectivity, rapid transmission, and acute onset. Traditional detection methods are time-consuming, have low sensitivity, and lack specificity, failing to meet the needs of rapid clinical diagnosis. Nucleic acid aptamers, as an emerging and innovative detection technology, offer novel solutions with high specificity, affinity, and broad target applicability, making them particularly promising for respiratory pathogen detection. This review highlights the progress in the research and application of nucleic acid aptamers for detecting respiratory pathogens, discussing their selection, application, potential in clinical diagnosis, and future development. Notably, these aptamers can significantly enhance the sensitivity and specificity of detection when combined with detection techniques such as fluorescence, colorimetry and electrochemistry. This review offers new insights into how aptamers can address the limitations of traditional diagnostic methods and advance clinical diagnostics. It also highlights key challenges and future research directions for the clinical application of nucleic acid aptamers.

Keywords Nucleic acid aptamers, Respiratory pathogens, Pathogen detection, SELEX, Clinical diagnosis

Introduction

Respiratory infections are a major global public health concern, particularly affecting vulnerable groups such as children, the elderly, and immunocompromised individuals. In 2019, lower respiratory infections (LRIs) caused approximately 2.5 million deaths globally, with a particularly severe impact on children under 5 and older adults [1]. Since the outbreak of COVID-19 in 2019, the threat posed by respiratory pathogens to human health has further intensified, highlighting the importance of rapid and accurate detection of these pathogens [2]. Respiratory pathogens include various viruses, bacteria, and fungi, such as influenza virus, novel coronavirus (SARS-CoV-2), *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis* [3, 4]. These pathogens are highly contagious and diverse, complicating efforts in disease prevention and control [5].

*Correspondence:
Yu Zhao
1458666138@qq.com
Hua-Wei Yi
yihuawei0902@126.com
[1]Laboratory Department, the First Affiliated Hospital of Yangtze University, Jingzhou, Hubei, People's Republic of China
[2]Health Science Center, Yangtze University, Jingzhou, Hubei, People's Republic of China
[3]Central Laboratory, the First Affiliated Hospital of Yangtze University, Jingzhou, Hubei, People's Republic of China
[4]Oncology Department, the First Affiliated Hospital of Yangtze University, Jingzhou, Hubei, People's Republic of China
[5]Laboratory Department, the People's Hospital of Yanhu District, Yuncheng, Shanxi, People's Republic of China

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Traditional pathogen detection methods include bacterial culture, immunoassays, and molecular biology techniques [6]. Bacterial culture, considered the gold standard, is time-consuming (typically requiring 48 h to several days), has high contamination risk [7], and is limited by the types of culture media available [8]. Immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and immunofluorescence detection, offer improved sensitivity and specificity but face challenges like poor antibody stability, cross-reactivity, and high production costs [9]. Molecular biology techniques, such as polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR), provide high sensitivity and specificity, becoming essential tools for pathogens detection [10, 11]. However, these techniques often require complex experimental procedures, sophisticated instruments, and high reagent costs, along with strict experimental conditions and sample quality requirements [12]. Metagenomic next-generation sequencing (mNGS) enables the direct detection of pathogens from clinical samples without the need for culture, offering comprehensive identification of microorganisms [13, 14]. However, mNGS is prone to false positives and negatives, and its broader application is limited by the lack of standardized methods, clear data interpretation guidelines, and external quality assessment procedures [15].

In recent years, nucleic acid aptamers have emerged as a promising new detection technology, offering unique advantages over traditional diagnostic methods. Aptamers are short DNA or RNA oligonucleotide sequences that exhibit high specificity and affinity for target molecules, with broad selectivity and ease of synthesis and modification in vitro [16, 17]. These attributes demonstrate their great potential in the field of pathogen detection. Nucleic acid aptamers can be rapidly obtained through systematic evolution of ligands by exponential enrichment (SELEX) technology, enabling efficient binding to target molecules [18]. Furthermore, the aptamers can be combined with various signal transduction systems, such as fluorescence labeling, electrochemical sensing, and surface plasmon resonance (SPR), further enhancing detection sensitivity and specificity [19, 20].

Extensive research has significantly expanded the application of nucleic acid aptamers in the detection of respiratory pathogens. For instance, nucleic acid aptamer detection technology for SARS-CoV-2 has played a crucial role in pandemic prevention and control, providing rapid, sensitive detection and portable devices for on-site testing, thereby greatly facilitating epidemic monitoring and control [21, 22]. These innovations highlight the unique advantage of aptamers in creating flexible and scalable solutions that address the limitations of traditional diagnostic methods. Furthermore, nucleic acid aptamer technology also shows broad potential in detection of influenza virus, respiratory syncytial virus, and *Streptococcus pneumoniae* and other pathogens, offering new perspectives for improving both the sensitivity and specificity of respiratory pathogen diagnostics [23, 24].

In conclusion, nucleic acid aptamers have great potential in advancing respiratory pathogen diagnostics. This mini review highlights the integration of aptamers with multiple detection technologies, including fluorescence, colorimetry, and electrochemistry, demonstrating their ability to enhance detection sensitivity and specificity. It underscores their innovative application in detecting respiratory pathogens, especially in urgent contexts like pandemic control. Furthermore, the review delves into the future potential of aptamers for rapid, on-site clinical diagnostics, illustrating their versatility and potential to address evolving healthcare challenges.

Selection of nucleic acid aptamers

Nucleic acid aptamers are short oligonucleotide sequences composed of single-stranded DNA or RNA, capable of binding to various target substances with high specificity and selectivity. In 1990, Ellington A.D. and Szostak J.W. introduced the concept of "aptamer" [25]. In the same year, Tuerk C. and Gold L. successfully selected nucleic acid aptamers that specifically bind to bacteriophage T4 DNA polymerase from a random RNA library [26]. Since then, numerous nucleic acid aptamers targeting various molecules have been researched.

Nucleic acid aptamers, often referred to as "chemical antibodies" due to their specific recognition capabilities similar to antibodies, offer significant advantages over traditional antibodies [27]. These advantages include high affinity and specificity, easy chemical modification, and good stability [28], making aptamers highly promising for extensive applications in fields such as disease diagnosis and treatment. Furthermore, through the strict selection and optimization process, the aptamers with higher affinity and specific binding to the target molecules can be screened, and their affinity can even reach the picomolar level [29]. In addition, the affinity can also be improved by designing a specific aptamer structure, such as hairpin structure and G-quadruple structure [30, 31]. Moreover, nucleic acid aptamers can be chemically modified (e.g., thiophosphate modification, lock-in nucleic acid modification) to enhance their stability and binding ability [32, 33]. By introducing specific chemical groups (e.g., fluorophores, biotin, and avidin) [32], and combining aptamers with PCR technology and nanotechnology, a series of methods can be developed to achieve rapid, efficient, and accurate detection of target molecules [34, 35].

Nucleic acid aptamers are typically selected through SELEX technology [36]. The key steps in this process include constructing a nucleic acid library with numerous

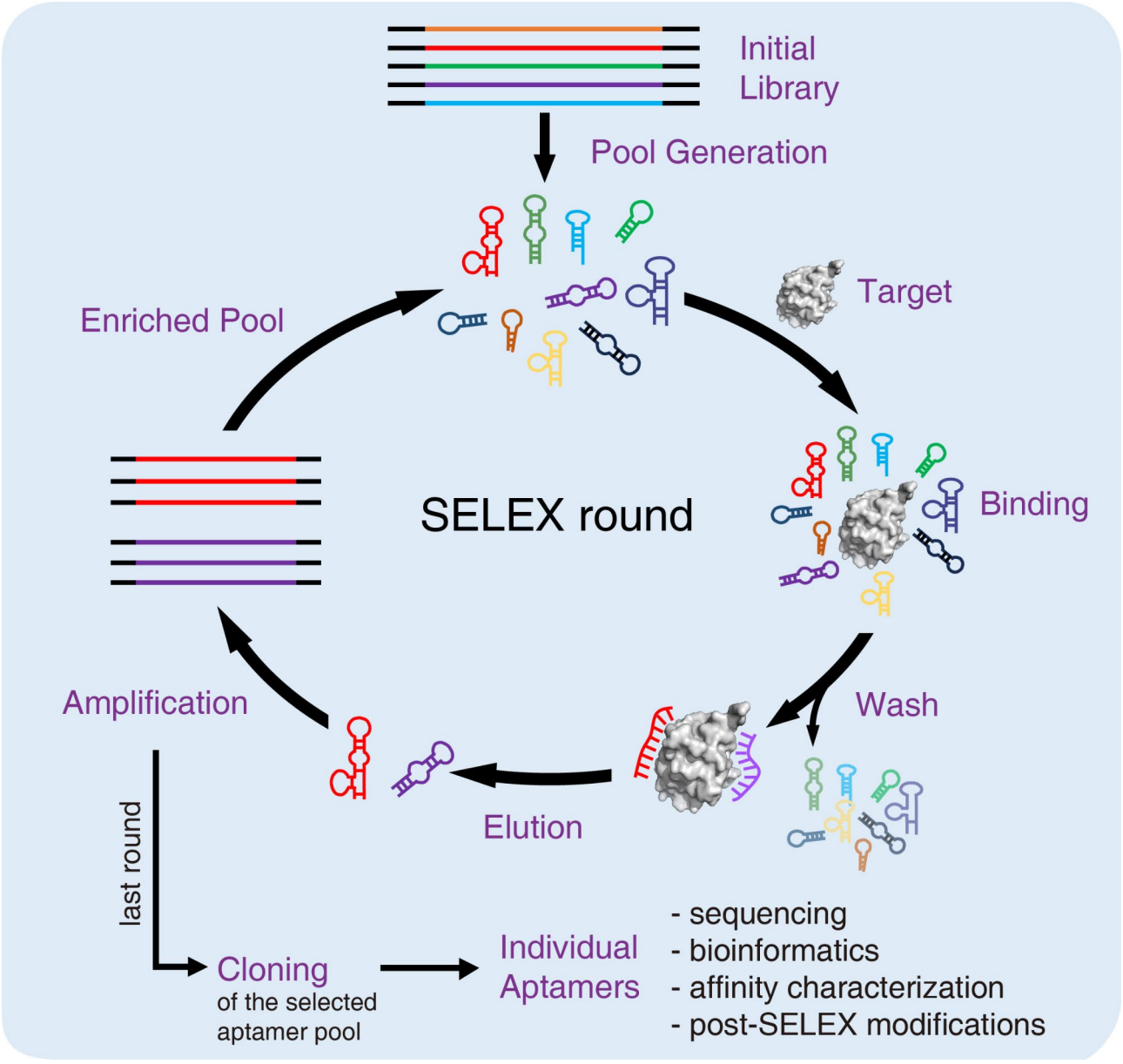

Fig. 1 Schematic diagram of SELEX technology

random sequences, binding the target molecule to the nucleic acid library, eluting unbound sequences, amplifying the bound sequences, and repeating these steps to enrich high-affinity, high-specificity aptamers [37], as shown in Fig. 1. In recent years, SELEX technology has made significant progress in selecting nucleic acid aptamers. Researchers have introduced new techniques, such as cell-SELEX and SELEX combined with high-throughput sequencing, which greatly enhance the efficiency and accuracy of nucleic acid aptamer selection [38, 39].

Aptamer-based detection technologies

Nucleic acid aptamers exhibit great potential in diagnostic fields due to their high specificity, sensitivity, and multifunctionality. To fully utilize these features, specific detection technologies are required to characterize their interactions with target molecules. Current aptamer-based detection technologies include various approaches, such as fluorescence aptamer probes, electrochemical aptamer sensors, colorimetric aptamer assays, surface plasmon resonance (SPR) aptamer sensors, lateral flow aptamer assays (LFAAs) [40]. Each method offers distinct advantages and is suited to specific application scenarios.

Fluorescence aptamer probes

Fluorescence aptamer probes detect changes in fluorescence intensity upon binding to target molecules [41]. The working principle primarily relies on fluorescence resonance energy transfer (FRET) and fluorescence quenching mechanisms [42]. These probes, labeled with fluorescent groups, bind to target molecules and induce conformational changes that alter the spatial orientation of the fluorescent labels, leading to changes in fluorescence signals. The fluorescent molecule in an aptamer may be activated or quenched upon target binding, producing measurable fluorescence signals. Fluorescence aptamer probes are known for their high sensitivity, selectivity, rapidity, a simple detection process, and a low price [43]. These probes have proven effective in identifying cancer markers, environmental pollutants, and various pathogens [44]. Studies have shown that optimizing aptamer sequences and fluorescent groups can further enhance the sensitivity and specificity of fluorescence aptamer probes [43].

Colorimetric aptamer assays

Colorimetric aptamer assays offer straightforward and intuitive detection methods by eliciting visible color changes in the presence of target molecules. These assays commonly utilize gold nanoparticles that aggregate upon encountering targets, leading to observable color shifts [45]. The intensity of these color changes can be quantitatively assessed using a spectrophotometer. Due to their simplicity and ease of use, these methods are particularly well-suited for settings with limited resources. Colorimetric aptamer assays are highly sensitive and specific, and have been extensively employed for the detection of toxins, bacteria, and viruses, offering swift and cost-effective diagnostic solutions [46, 47]. Research indicates that the sensitivity and specificity of colorimetric aptamer assays can be enhanced by optimizing the size and surface characteristics of gold nanoparticles [48]. Additionally, integrating these assays with other detection technologies, such as magnetic separation and signal amplification, can substantially improve both the accuracy and reliability of the detection process [48].

Surface plasmon resonance aptamer sensors

Surface plasmon resonance (SPR) is a label-free detection method that monitors changes in the refractive index near the sensor surface as aptamers bind to target molecules [49]. This method is based on the principle that light striking a metal surface excites surface plasmon waves. When target molecules interact with aptamers on the sensor surface, the resulting shift in refractive index modifies the SPR resonance angle, which can be continuously monitored and recorded. SPR aptamer sensors facilitate real-time observation of interactions without the need for additional labels. This technology is highly sensitive and capable of detecting a broad spectrum of biomolecules, and have been utilized in drug discovery, clinical diagnostics, and environmental monitoring [50, 51]. Moreover, as the technology progresses, the performance and application scope of SPR aptamer sensors are continually broadening. For instance, the integration of microfluidics with SPR aptamer sensors allows for high-throughput screening of multiple targets [52].

Lateral flow aptamer assays

Lateral flow aptamer assays (LFAAs) use aptamers as recognition elements, similar to traditional lateral flow immunoassays, providing a portable and user-friendly platform for rapid diagnostics. The test strip facilitates sample flow via capillary action, where aptamers interact with target molecules to produce visible color changes, simplifying operation and result interpretation [53]. LFAAs are especially well-suited for resource-limited settings and field testing due to their simplicity and rapid results. They are extensively employed for detecting infectious diseases, foodborne pathogens, and clinical markers [54]. Additionally, the development of multifunctional LFAAs is an ongoing trend. Designing test strips with multiple channels allows for the simultaneous detection of various target molecules, offering comprehensive diagnostic insights. Integrating LFAAs with portable devices like smartphones also supports the digitalization and remote transmission of results, significantly enhancing their convenience and practicality [55, 56].

Electrochemical aptamer sensors

Electrochemical aptamer sensors identify target molecules by tracking alterations in electrical signals following the binding of aptamers to their targets. These sensors function on the principle that such bindings result in discernible changes in electrical signals. By assessing variations in current, voltage, or impedance, the presence of the target can be quantified [57]. The precise nature of aptamer-target interactions enables these sensors to achieve highly accurate detections. Electrochemical aptamer sensors are celebrated for their high sensitivity, swift response, and potential for miniaturization, which renders them ideal for point-of-care diagnostics. They have been utilized to detect pathogens in food, glucose levels in blood, and heavy metals in water [58, 59]. Furthermore, these sensors can be incorporated into portable devices for on-the-spot detection [60].

Application of aptamers in the detection of respiratory pathogens

Application of nucleic acid aptamers in virus detection

Nucleic acid aptamers demonstrate substantial potential in virus detection, with specific capabilities for recognizing various respiratory viruses such as influenza virus, respiratory syncytial virus, and coronaviruses, as detailed in Table 1. For instance, aptamers that target the H1N1 influenza virus have been successfully identified and utilized for rapid on-site detection [61, 62].

Influenza virus

Influenza viruses are part of the Orthomyxoviridae family and possess segmented single-stranded negative-sense RNA genomes [70]. They are classified into types A, B, C, and D based on the antigenicity of their nucleoprotein and matrix protein, with types A and B being the main pathogens responsible for human influenza [71]. Since the 20th century, at least three major influenza pandemics have occurred, collectively resulting in approximately 100 million deaths. Annually, the WHO reports that around 250,000 people succumb to seasonal influenza. Due to considerable antigenic drift and shift, the influenza A virus continues to be a formidable pathogen [72], highlighting the critical need for ongoing influenza prevention and control efforts.

Researchers have identified multiple aptamers targeting influenza viruses, with the majority aimed at influenza A. Bharadwaj et al. isolated aptamers that recognize the influenza A H1N1 virus by targeting the mini-HA (the stable stem region of hemagglutinin) and the complete H1N1 virus. They developed an electrochemical aptamer sensor capable of rapidly differentiating H1N1 from other influenza A subtypes, achieving a limit of detection (LOD) of 3.7 PFU/mL [63]. Tseng et al. developed an integrated microfluidic system and constructed a sandwich-based aptamer assay on this platform, which detects the H1N1 virus within 30 min. This approach significantly increases sensitivity, making it 10^3 times higher than traditional serological assays [64].

Respiratory syncytial virus

Respiratory syncytial virus (RSV), a prevalent respiratory pathogen within the paramyxoviridae family, is characterized as a single-stranded negative-sense RNA virus [73]. RSV is notorious for causing respiratory infections primarily in infants, the elderly, and immunocompromised individuals, with children under five being particularly vulnerable [74, 75]. Infections may lead to a variety of respiratory symptoms, and in severe cases, can escalate to pneumonia, bronchitis, and recurrent wheezing, significantly affecting children's health. Thus, prompt and precise detection of RSV is imperative for effective disease management and control.

Aptamers, serving as innovative biological recognition elements, exhibit substantial promise for RSV detection. Szakács et al. utilized fluorescent aptamers to specifically label RSV attachment glycoprotein (G), enabling single-particle tracking analysis to identify, classify, and count individual RSV particles, successfully detecting single RSV particles in diluted throat swab samples [76]. Percze et al. developed aptamers that enhance the diagnostic sensitivity and specificity for detecting RSV, refining the SELEX process to streamline the aptamer selection [67]. Their research confirmed the aptamers' stability and efficacy in complex samples, underscoring their potential in clinical diagnostics.

Coronaviruses

Coronaviruses are enveloped, single-stranded positive-sense RNA viruses known to induce a spectrum of respiratory ailments, ranging from mild to severe. The novel coronavirus (SARS-CoV-2), a member of the beta-coronavirus subgroup, is responsible for severe acute respiratory syndrome and is highly infectious [77]. According to data from the World Health Organization, as of August 2024, the total number of confirmed cases worldwide has reached 770 million, resulting in 7 million cumulative deaths, prompting significant global concern and health emergencies [78]. Rapid and accurate detection methods are urgently needed for large-scale screening of SARS-CoV-2 to identify early infections and asymptomatic carriers, thereby preventing the spread of SARS-CoV-2.

Table 1 Aptamers for the detection of respiratory virus

Organism	Target	Method	Affinity (K_D)	DL	Ref.
H1N1	mini-HA and whole virus	electrochemistry	19.2 nM	3.7 pfu/mL	[63]
H1N1	whole virus	fluorescence	55.14 ± 22.4 nM	0.032 HAU	[64]
H1N1	whole virus	fluorescence	/	138 pg/mL	[65]
Influenza B	NPS	colorimetry	0.97 ± 0.6 nM	0.16 pg/mL	[66]
RSV	glycoproteins	fluorescence	/	/	[67]
SARS-COV-2	RBD	electrochemistry	/	66 pg/mL	[68]
SARS-COV-2	spike proteins	LFAAS	2–10 nM	91.2ng/ml	[69]

DL: Detection limit, HAU: hemagglutination unit, Ref: references

Aptamers, with their unique properties, show significant potential for applications in COVID-19 detection [68].

Ban et al. developed a DNA aptamer-coupled graphene field-effect transistor (GFET) biosensor platform for detecting SARS-CoV-2 and its variants in saliva samples, achieving rapid and accurate results within 20 min [79]. Li et al. introduced a gold nanoparticle-based lateral flow assay that uses aptamers for the visual detection of the wild-type SARS-CoV-2 spike protein (SPS) and its variants, achieving a detection limit of 0.68 nM [69]. Wu et al. created an automated integrated microfluidic system employing novel SELEX-selected SARS-CoV-2 aptamers for detecting inactivated SARS-CoV-2, eight SARS-CoV-2 pseudoviruses, and clinical isolates of SARS-CoV-2 [80]. Aptamer-based detection technologies are providing innovative solutions for the rapid, efficient, and convenient monitoring of SARS-CoV-2 and its variants, essential for public health and environmental safety. These technologies hold great promise for the rapid screening and diagnosis of SARS-CoV-2.

Application of nucleic acid aptamers in bacterial detection

In addition to viruses, nucleic acid aptamers have garnered attention for bacterial detection, enabling the identification of various pathogenic bacteria, including *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae* and so on, as shown in Table 2. The high specificity and sensitivity of aptamers make them essential tools for the early diagnosis of bacterial infections [81].

Streptococcus pneumoniae

Streptococcus pneumoniae, a gram-positive bacterium, was first isolated from patient sputum by Pasteur and Sternberg in 1881 [88]. It typically resides in the human nasopharynx and can become pathogenic when the immune system is compromised. *S. pneumoniae* is a primary cause of community-acquired pneumonia and can also cause meningitis, otitis media, and sepsis, all of which have high morbidity and mortality rates [89]. In 2019, *S. pneumoniae* was responsible for over 600,000 deaths [90]. Therefore, the rapid and early identification of *S. pneumoniae* is crucial for reducing mortality.

Zhang et al. established a novel and highly sensitive method for simultaneous detection of *S. pneumoniae* and *S. aureus* [32]. This method utilizes nucleic acid aptamer nanoprobes mediated by a hairpin allosteric mechanism and an aptamer-assisted CRISPR system, achieving a detection limit of 135 cfu/mL and a reduced detection time of 55 min [32]. Bayrac et al. selected aptamers targeting *S. pneumoniae* through bacterial SELEX and integrated these aptamers into a graphene oxide-based fluorescent assay for live cell detection, which also inhibits biofilm formation [24]. These methods provide efficient, rapid, and specific detection of *S. pneumoniae*, thereby improving diagnostic accuracy and offering new avenues for antimicrobial drug development.

Mycobacterium tuberculosis

Mycobacterium tuberculosis, the causative agent of tuberculosis, primarily infects the lungs but can also affect other organs [91]. The global tuberculosis epidemic has been exacerbated by factors such as HIV prevalence, drug-resistant strains, immunosuppressive drugs, drug abuse, and population movements. HIV-infected individuals with *M. tuberculosis* are at a heightened risk of developing active tuberculosis, increasing AIDS-related morbidity and mortality. In 2020, an estimated 1.3 million people died from tuberculosis [92]. Thus, developing novel biomolecules for early detection of *M. tuberculosis* is critical. Aptamers, with their unique properties, have been widely utilized to construct highly sensitive detection platforms for the early and accurate detection of *M. tuberculosis*.

Azmi et al. combined the *M. tuberculosis* biomarker (CFP10-ESAT6 antigen) with aptamers on a graphene/polyaniline (GP/PANI) modified gold electrode to form a sandwich-type portable electrochemical biosensor, enhancing signal amplification and achieving a detection limit of 1.5 ng/mL [84]. Das et al. introduced methylene blue into an aptamer electrochemical biosensor, detecting the HSPX antigen in cerebrospinal fluid samples within 30 min [93]. Li et al. combined aptamers with

Table 2 Aptamers for the detection of respiratory bacteria

Organism	target	Method	Affinity (K_D)	DL	Ref.
S. pneumoniae	whole bacterium	fluorescence	661.8 ± 111.3 nM	15 cfu/mL	[21]
S. aureus	whole bacterium	electrochemistry	16.5 ± 3.4 nM	39 cfu /mL	[82]
	whole bacterium	electrochemistry	14.47 ± 8.18 nM	414 cfu /mL	
S. aureus	whole bacterium	colorimetric	/	8 cfu /mL	[83]
M. tuberculosis	CFP10-ESAT6	electrochemistry	/	1.5 ng/mL	[84]
P. aeruginosa	LPS	fluorescence	46.2 ± 9.5 nM	88 ng/mL	[85]
P. aeruginosa	3-O-C_{12}-HSL	electrochemistry	106.7 nM	145 ng/mL	[86]
K. pneumoniae	whole bacterium	SPR	/	3.4×10^3 cfu /mL	[87]

DL: Detection limit, LPS: Lipopolysaccharide, Ref: references

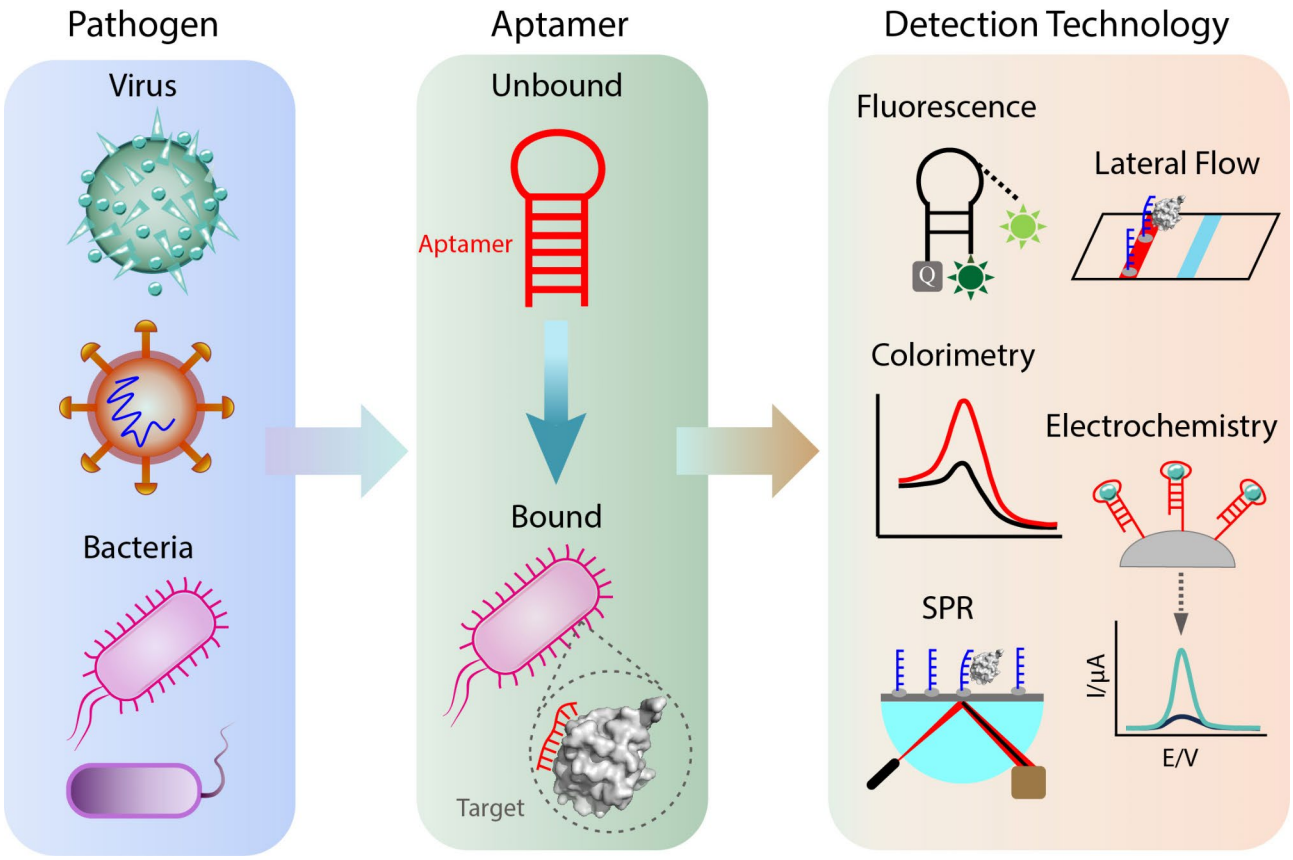

Fig. 2 Schematic diagram of aptamer detection for respiratory pathogens

DNA click ligation technology for ultrasensitive detection of the CFP-10 antigen, with a detection limit as low as 10 pg/mL [94]. Aptamer biosensors are portable, easy to operate, and suitable for rapid on-site detection, enhancing early tuberculosis control and management.

Klebsiella pneumoniae

Klebsiella pneumoniae, a common gram-negative bacterium, belongs to the Enterobacteriaceae family [95]. It typically resides in the upper respiratory tract and intestines of healthy individuals and is usually non-pathogenic. However, under certain conditions such as immune system weakening or dysbiosis, it can cause various infectious diseases including pneumonia, peritonitis, meningitis, urinary tract infections, and sepsis, posing a significant risk in hospital-acquired infections [96]. In recent years, the increasing prevalence of carbapenem-resistant *K. pneumoniae* has presented a significant challenge to clinical treatment. Early, accurate, and rapid detection of *K. pneumoniae* is crucial for disease control and treatment guidance.

Deb et al. designed a plasmonic aptamer-gold nanoparticle (Au-NP) sensor based on the localized surface plasmon resonance (LSPR) phenomenon of Au-NPs [87]. When *K. pneumoniae* binds to the aptamer sensor, it alters the charge distribution and refractive index on the Au-NP surface, resulting in changes in the LSPR spectrum, enabling highly specific and sensitive detection of *K. pneumoniae* [87]. This method supports early diagnosis and treatment, and aptamers' ease of operation and cost-effectiveness offer broad application prospects, showcasing significant potential in *K. pneumoniae* detection.

Application of nucleic acid aptamers in multiplex infection detection

Respiratory infections often involve co-infections with multiple pathogens. Nucleic acid aptamers, due to their high specificity and sensitivity, can simultaneously detect multiple pathogens, offering a novel approach for diagnosing multiplex infections. Multiplex aptamer sensors and microarray technologies are effective means to achieve this goal.

Song et al. used the sequential toggle cell-SELEX (STC-SELEX) method to isolate highly affine aptamers targeting bacteria of different genera, enabling simultaneous detection of six pathogens including *E. coli*, *K. pneumoniae*, *S. epidermidis* and others using fluorescent-labeled aptamers [97]. Kukushkin et al. used multiple lithography techniques to fabricate a surface-enhanced

Raman spectroscopy (SERS) substrate with four active regions, each modified with a different labeled aptamer [98]. This multiple lithography SERS aptamer sensor allowed for the simultaneous detection of RSV, influenza A virus, adenovirus, and SARS-CoV-2 within 17 min [98]. Effah et al. employed vancomycin-modified polyimine-interlayer superparamagnetic iron oxy-gold nanoparticles and aptamer-conjugated surface-enhanced Raman scattering as capture probes for broad-spectrum bacteria and specific probes for target bacteria, respectively [99]. They further developed a sensor with a dual recognition strategy, successfully achieving the co-detection of *K. pneumoniae* and *A. baumannii* [99]. This specific and efficient method greatly facilitates simultaneous research on multiple pathogens, elucidating their interrelationships and enhancing protection of human health.

Challenges in clinical application of nucleic acid aptamers

Nucleic acid aptamers, as innovative biomolecules, demonstrate considerable potential for clinical applications in disease diagnosis and treatment. Employing detection methods such as fluorescence, colorimetric analysis, and electrochemical sensing, these aptamers can identify a range of respiratory pathogens, including bacteria and viruses, marking significant advances in laboratory research, as shown in Fig. 2. However, several challenges must be addressed for clinical application.

Each detection method has its own limitations. For instance, fluorescence aptamer probes can suffer from high background signals, reducing accuracy in complex samples, while colorimetric assays, though simple and cost-effective, may have lower sensitivity and be influenced by sample color or turbidity. Surface plasmon resonance sensors offer high sensitivity but require expensive equipment and complex analysis. Lateral flow assays are portable and user-friendly but typically provide qualitative results and may lack the sensitivity for low-concentration pathogens. Electrochemical sensors, while sensitive, often need controlled conditions and specialized equipment, limiting their point-of-care use. Therefore, selecting the appropriate method for pathogen detection depends on factors such as pathogen type, required sensitivity, available resources, and testing environment to ensure optimal performance.

In addition to the challenges with detection methods, aptamer structures are sensitive to environmental conditions such as temperature, pH, and ionic strength, which can influence their binding affinity and specificity. To ensure reliable clinical applications, it is crucial to enhance their stability through advanced chemical modifications. Unmodified aptamers are vulnerable to nuclease enzyme attack in vivo, resulting in a shortened half-life and seriously affecting their stability [100]. Nucleic acid aptamers can be chemically modified to enhance their stability and binding ability. In addition, producing high-affinity aptamers often involves a complex and repeated SELEX screening process, posing a barrier to mass production. Simplifying and automating this process, along with improving production efficiency and standardizing the selection, modification, and purification steps, are essential for enhancing scalability and consistency [101]. Additionally, nonspecific binding in complex biological samples can reduce detection accuracy, making the development of new aptamer designs and effective blocking strategies a critical research challenge [102].

Despite these challenges, the distinctive advantages of nucleic acid aptamers render them invaluable in modern diagnostics. Addressing these challenges through ongoing innovation and interdisciplinary collaboration will facilitate their successful clinical integration, ultimately improving global health outcomes.

Summary and outlook

Nucleic acid aptamers offer unmatched specificity, low immunogenicity, and ease of modification, making them highly promising for pathogen detection. Compared to traditional methods like PCR and ELISA, aptamers provide faster detection, easier synthesis, and high specificity, as shown in Table 3. Despite challenges like complex screening and stability issues, advancements in selection technologies and expanding applications suggest aptamers will play a key role in diagnosing and treating respiratory infections.

The outlook of nucleic acid aptamers in respiratory pathogen detection is exceptionally promising. Continuous improvements in technology will expand their clinical applications. Future research should focus on optimizing aptamer design, enhancing stability through chemical modifications, and exploring innovative materials. High-throughput screening and automated synthesis will be crucial for improving efficiency, while addressing standardization and developing diverse platforms will support faster, more precise detection.

Moreover, the integration of nucleic acid aptamers with cutting-edge technologies such as microfluidics, nanotechnology, and advanced signal amplification methods can significantly improve detection sensitivity and specificity. The creation of portable, easy-to-use diagnostic tools based on aptamers will support rapid, on-site pathogen detection, which is particularly valuable in resource-limited settings and during infectious disease outbreaks.

In conclusion, nucleic acid aptamers are transformative in pathogen detection. Their unique properties and versatility position them as essential tools in modern

Table 3 Comparative analysis of diagnostic methods for respiratory pathogens

Methods	Advantages	Drawbacks / Limitations	Reference
Culture	Low cost; Suitable for many sample types; Allows drug susceptibility testing	Time-consuming; High contamination risk; Low sensitivity, especially with antibiotics; Limited by culture medium type	[7, 8] [103]
ELISA	High sensitivity and specificity;	Prone to non-specific interference; High production cost; Possible false negatives; Complex operation	[9] [104] [105]
PCR	High sensitivity and specificity; Time-efficient	Risk of false positives/negatives; Cumbersome process; Requires sophisticated instruments and high-cost reagents; Strict conditions needed	[7] [10–12] [106]
mNGS	Direct sequencing without culture; Unbiased, broad-spectrum detection	Prone to false positives/negatives; Complex, high-cost operation; Lack of standardized methods and guidelines; No dedicated quality control	[13–15]
Aptamer	High specificity and affinity; Easy to synthesize; Time-efficient	Complex screening and preparation; Poor internal stability; Lack of standardized procedures; Screening methods need optimization	[16, 17] [27]

diagnostics and therapeutic interventions, with the potential to significantly improve global health outcomes.

Author contributions
Conceptualization, R.-M.F. and H.-W.Y.; investigation, R.-M.F., Y.Z. and H.-W.Y.; visualization, R.-M.F. and H.-W.Y.; writing—original draft preparation, R.-M.F., Y.L., Z.-Q.L., L.W., Y.Z. and H.-W.Y.; writing—review and editing, N.C., R.-M.F., Y.Z. and H.-W.Y. All authors have read and agreed to the published version of the manuscript.

Funding
This work was supported by Jingzhou joint science and technology fund project, Hubei Province, China [No. 2024LHY13 (to H.-W.Y.)] and the doctoral research start-up fund project of the First People's Hospital of Jingzhou, China [No. 2023DIF03 (to H.-W.Y.)].

Data availability
No datasets were generated or analysed during the current study.

Declarations

Institutional Review Board Statement
Not applicable.

Informed consent
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 28 July 2024 / Accepted: 21 September 2024
Published online: 30 September 2024

References

1. Li Y, Nair H. Trends in the global burden of lower respiratory infections: the knowns and the unknowns. Lancet Infect Dis. 2022;22(11):1523–5.
2. Zhao H, Yang Y, Lyu J, Ren X, Cheng W. Development and application of a method to detect 27 respiratory pathogens using multiplex RT-PCR combined with MassARRAY technology. BMC Infect Dis. 2021;21(1):870.
3. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd, Kato T, Lee RE, et al. SARS-CoV-2 Reverse Genetics reveals a variable infection gradient in the respiratory tract. Cell. 2020;182(2):429–46.
4. Li Z-J, Zhang H-Y, Ren L-L, Lu Q-B, Ren X, Zhang C-H, Wang Y-F, Lin S-H, Zhang X-A, Li JJ. Etiological and epidemiological features of acute respiratory infections in China. Nat Commun. 2021;12(1):5026.
5. Babawale PI, Guerrero-Plata A. Respiratory viral coinfections: insights into Epidemiology, Immune Response, Pathology, and clinical outcomes. Pathogens. 2024;13(4):316–28.
6. Kabiraz MP, Majumdar PR, Mahmud MC, Bhowmik S, Ali A. Conventional and advanced detection techniques of foodborne pathogens: a comprehensive review. Heliyon. 2023;9(4):e15482.
7. Gerace E, Mancuso G, Midiri A, Poidomani S, Zummo S, Biondo C. Recent advances in the use of molecular methods for the diagnosis of bacterial infections. Pathogens. 2022;11(6):663.
8. Davydova A, Vorobjeva M, Pyshnyi D, Altman S, Vlassov V, Venyaminova A. Aptamers against pathogenic microorganisms. Crit Rev Microbiol. 2016;42(6):847–65.
9. Hayrapetyan H, Tran T, Tellez-Corrales E, Madiraju C. Enzyme-linked immunosorbent assay: types and applications. Methods Mol Biol. 2023;2612:1–17.
10. Liu Q, Jin X, Cheng J, Zhou H, Zhang Y, Dai Y. Advances in the application of molecular diagnostic techniques for the detection of infectious disease pathogens. Mol Med Rep. 2023;27(5):104.
11. Yi H-W, Wang X-M, Tan X, Ding C-Z, Zhang C-L, Wu J-H, Li Q, Xin C-Q, Fan W. Simultaneous detection of human norovirus GI, GII and SARS-CoV-2 by a quantitative one-step triplex RT-qPCR. Front Microbiol. 2024;14:1269275.
12. Sanders R, Mason DJ, Foy CA, Huggett JF. Considerations for accurate gene expression measurement by reverse transcription quantitative PCR when analysing clinical samples. Anal Bioanal Chem. 2014;406(26):6471–83.
13. Edward P, Handel AS. Metagenomic next-generation sequencing for infectious Disease diagnosis: a review of the literature with a focus on pediatrics. J Pediatr Infect Dis Soc. 2021;10(Supplement_4):71–7.
14. Hao J, Li W, Wang Y, Zhao J, Chen Y. Clinical utility of metagenomic next-generation sequencing in pathogen detection for lower respiratory tract infections and impact on clinical outcomes in southernmost China. Front Cell Infect Microbiol. 2023;13:1271952.
15. Tsang HF, Yu ACS, Jin N, Yim AKY, Leung WMS, Lam KW, et al. The clinical application of metagenomic next-generation sequencing for detecting pathogens in bronchoalveolar lavage fluid: case reports and literature review. Expert Rev Mol Diagn. 2022;22(5):575–82.
16. Hu PP. Recent advances in Aptamers Targeting Immune System. Inflammation. 2017;40(1):295–302.
17. Li H-Y, Jia W-N, Li X-Y, Zhang L, Liu C, Wu J. Advances in detection of infectious agents by aptamer-based technologies. Emerg Microbes Infect. 2020;9(1):1671–81.
18. Duan Y, Zhang C, Wang Y, Chen G. Research progress of whole-cell-SELEX selection and the application of cell-targeting aptamer. Mol Biol Rep. 2022;49(8):7979–93.
19. Villalonga A, Pérez-Calabuig AM, Villalonga R. Electrochemical biosensors based on nucleic acid aptamers. Anal Bioanal Chem. 2020;412(1):55–72.
20. Léguillier V, Heddi B, Vidic J. Recent advances in Aptamer-based biosensors for bacterial detection. Biosens (Basel). 2024;14(5):210.
21. Chen Z, Wu Q, Chen J, Ni X, Dai J. A DNA aptamer based method for detection of SARS-CoV-2 nucleocapsid protein. Virol Sin. 2020;35(3):351–4.
22. Song Y, Song J, Wei X, Huang M, Sun M, Zhu L, Lin B, Shen H, Zhu Z, Yang C. Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein. Anal Chem. 2020;92(14):9895–900.
23. Wang M, Hao M-C, Huangfu Y, Yang K-Z, Zhang X-Q, Zhang Y, Chen J, Zhang Z-L. A Universal Aptamer for Influenza A viruses: selection, Recognition, and infection inhibition. ACS Pharmacol Transl Sci. 2023;7(1):249–58.
24. Bayraç AT, Donmez SI. Selection of DNA aptamers to Streptococcus pneumonia and fabrication of graphene oxide based fluorescent assay. Anal Biochem. 2018;556:91–8.

25. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990;346(6287):818–22.
26. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249(4968):505–10.
27. Byun J. Recent Progress and opportunities for Nucleic Acid aptamers. Life (Basel). 2021;11(3):193.
28. Zhou J, Rossi J. Aptamers as targeted therapeutics: current potential and challenges. Nat Rev Drug Discov. 2017;16(3):181–202.
29. Ştefan G, Hosu O, De Wael K, Lobo-Castañón MJ, Cristea C. Aptamers in biomedicine: selection strategies and recent advances. Electrochim Acta. 2021;376:137994.
30. Zhang L, Xu X, Cao L, Zhu Z, Ding Y, Jiang H, Li B, Liu J. Multi-aptamer-mediated hairpin allosteric and aptamer-assisted CRISPR system for detection of S. pneumoniae and S. Aureus. Mikrochim Acta. 2023;191(1):29.
31. Gupta A, Anand A, Jain N, Goswami S, Anantharaj A, Patil S, Singh R, Kumar A, Shrivastava T, Bhatnagar S, et al. A novel G-quadruplex aptamer-based spike trimeric antigen test for the detection of SARS-CoV-2. Mol Ther-Nucl Acids. 2021;26:321–32.
32. Wu X, Chen J, Wu M, Zhao JX. Aptamers: active targeting ligands for cancer diagnosis and therapy. Theranostics. 2015;5(4):322–44.
33. Ni X, Castanares M, Mukherjee A, Lupold SE. Nucleic acid aptamers: clinical applications and promising new horizons. Curr Med Chem. 2011;18(27):4206–14.
34. Pinto A, Bermudo Redondo MC, Ozalp VC, O'Sullivan CK. Real-time apta-PCR for 20 000-fold improvement in detection limit. Mol Biosyst. 2009;5(5):548–53.
35. Wang Z, Fang X, Sun N, Deng C. A rational route to hybrid aptamer-molecularly imprinted magnetic nanoprobe for recognition of protein biomarkers in human serum. Anal Chim Acta. 2020;1128:1–10.
36. Mayer G. The chemical biology of aptamers. Angew Chem Int Ed Engl. 2009;48(15):2672–89.
37. Stoltenburg R, Reinemann C, Strehlitz B. SELEX—A (r) evolutionary method to generate high-affinity nucleic acid ligands. Biomol Eng. 2007;24(4):381–403.
38. Sefah K, Shangguan D, Xiong X, O'donoghue MB, Tan W. Development of DNA aptamers using Cell-SELEX. Nat Protoc. 2010;5(6):1169–85.
39. Komarova N, Barkova D, Kuznetsov A. Implementation of high-throughput sequencing (HTS) in Aptamer Selection Technology. Int J Mol Sci. 2020;21(22):8774.
40. Teng J, Yuan F, Ye Y, Zheng L, Yao L, Xue F, Chen W, Li B. Aptamer-Based technologies in Foodborne Pathogen Detection. Front Microbiol. 2016;7:1426.
41. Wang RE, Zhang Y, Cai J, Cai W, Gao T. Aptamer-based fluorescent biosensors. Curr Med Chem. 2011;18(27):4175–84.
42. Redy O, Kisin-Finfer E, Sella E, Shabat D. A simple FRET-based modular design for diagnostic probes. Org Biomol Chem. 2012;10(4):710–5.
43. Lee M, Shin S, Kim S, Park N. Recent advances in Biological Applications of Aptamer-based fluorescent biosensors. Molecules. 2023;28(21):7327.
44. Nguyen VT, Kwon YS, Gu MB. Aptamer-based environmental biosensors for small molecule contaminants. Curr Opin Biotechnol. 2017;45:15–23.
45. Vishwakarma A, Lal R, Ramya M. Aptamer-based approaches for the detection of waterborne pathogens. Int Microbiol. 2021;24(2):125–40.
46. Luan Y, Chen J, Li C, Xie G, Fu H, Ma Z, Lu A. Highly sensitive colorimetric detection of Ochratoxin A by a label-free aptamer and gold nanoparticles. Toxins (Basel). 2015;7(12):5377–85.
47. Das R, Dhiman A, Kapil A, Bansal V, Sharma TK. Aptamer-mediated colorimetric and electrochemical detection of Pseudomonas aeruginosa utilizing peroxidase-mimic activity of gold NanoZyme. Anal Bioanal Chem. 2019;411(6):1229–38.
48. Liu J, Lu Y. Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles. Angew Chem Int Ed Engl. 2005;45(1):90–4.
49. Chang CC. Recent advancements in Aptamer-based surface Plasmon Resonance Biosensing Strategies. Biosens (Basel). 2021;11(7):233.
50. Homola J. Surface plasmon resonance sensors for detection of chemical and biological species. Chem Rev. 2008;108(2):462–93.
51. Masson JF. Surface Plasmon Resonance Clinical biosensors for Medical Diagnostics. ACS Sens. 2017;2(1):16–30.
52. Weng CH, Huang CJ, Lee GB. Screening of aptamers on microfluidic systems for clinical applications. Sens (Basel). 2012;12(7):9514–29.
53. Yang LF, Kacherovsky N, Panpradist N, Wan R, Liang J, Zhang B, et al. Aptamer Sandwich lateral Flow Assay (AptaFlow) for antibody-free SARS-CoV-2 detection. Anal Chem. 2022;94(20):7278–85.
54. Majdinasab M, Badea M, Marty JL. Aptamer-based lateral Flow assays: current trends in Clinical Diagnostic Rapid tests. Pharmaceuticals (Basel). 2022;15(1):90.
55. Bahadır EB, Sezgintürk MK. Lateral flow assays: principles, designs and labels. Trac-Trend Anal Chem. 2016;82:286–306.
56. Rodriguez NM, Wong WS, Liu L, Dewar R, Klapperich CM. A fully integrated paperfluidic molecular diagnostic chip for the extraction, amplification, and detection of nucleic acids from clinical samples. Lab Chip. 2016;16(4):753–63.
57. Radi A-E. Electrochemical Aptamer-based biosensors: recent advances and perspectives. Int J Electrochem. 2011;2011:863196.
58. Appaturi JN, Pulingam T, Thong KL, Muniandy S, Ahmad N, Leo BF. Rapid and sensitive detection of Salmonella with reduced graphene oxide-carbon nanotube based electrochemical aptasensor. Anal Biochem. 2020;589:113489.
59. Ge S, Ma X. Electrochemical aptamer-based sensors for the detection of heavy metals. Int J Electrochem SC. 2022;17:220926.
60. Chen B, Wang D, Wei S, Wang J. Portable electrochemical aptasensor for highly sensitive detection of 3,3',4,4'-tetrachlorobiphenyl. Biosens Bioelectron. 2024;260:116434.
61. Zou X, Wu J, Gu J, Shen L, Mao L. Application of aptamers in Virus Detection and antiviral therapy. Front Microbiol. 2019;10:1462.
62. Kim SM, Kim J, Noh S, Sohn H, Lee T. Recent development of Aptasensor for Influenza Virus Detection. Biochip J. 2020;14(4):327–39.
63. Bhardwaj J, Chaudhary N, Kim H, Jang J. 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.
64. Tseng YT, Wang CH, Chang CP, Lee GB. Integrated microfluidic system for rapid detection of influenza H1N1 virus using a sandwich-based aptamer assay. Biosens Bioelectron. 2016;82:105–11.
65. Lee N, Wang C, Park J. User-friendly point-of-care detection of influenza A (H1N1) virus using light guide in three-dimensional photonic crystal. RSC Adv. 2018;8(41):22991–7.
66. Kang J, Yeom G, Jang H, Park CJ, Kim MG. Highly sensitive and universal detection strategy based on a colorimetric assay using target-specific heterogeneous sandwich DNA aptamer. Anal Chim Acta. 2020;1123:73–80.
67. Percze K, Szakács Z, Scholz É, András J, Szeitner Z, Kieboom CH, Ferwerda G, Jonge MI, Gyurcsányi RE, Mészáros T. Aptamers for respiratory syncytial virus detection. Sci Rep. 2017;7:42794.
68. Abrego-Martinez JC, Jafari M, Chergui S, Pavel C, Che D, Siaj M. Aptamer-based electrochemical biosensor for rapid detection of SARS-CoV-2: Nanoscale electrode-aptamer-SARS-CoV-2 imaging by photo-induced force microscopy. Biosens Bioelectron. 2022;195:113595.
69. Li X, Wang J, Yang G, Fang X, Zhao L, Luo Z, Dong Y. The development of Aptamer-based gold nanoparticle lateral Flow Test strips for the detection of SARS-CoV-2 S proteins on the Surface of Cold-Chain Food Packaging. Molecules. 2024;29(8):1776.
70. Mubareka S, Palese P. Influenza virus: the biology of a changing virus. Influenza Vaccines Future. 2011: 3–26.
71. Yamayoshi S, Kawaoka Y. Current and future influenza vaccines. Nat Med. 2019;25(2):212–20.
72. Schweiger B, Zadow I, Heckler R. Antigenic drift and variability of influenza viruses. Med Microbiol Immunol. 2002;191(3–4):133–8.
73. Taleb SA, Al Thani AA, Al Ansari K, Yassine HM. Human respiratory syncytial virus: pathogenesis, immune responses, and current vaccine approaches. Eur J Clin Microbiol Infect Dis. 2018;37(10):1817–27.
74. Krilov LR, Roberts NJ Jr. Respiratory syncytial virus (RSV) update. Viruses. 2022;14(10):2110.
75. Roberts NJ Jr. Respiratory syncytial virus suppression of the antiviral immune response: implications for evaluation of candidate vaccines. Vaccine. 2019;37(51):7451–4.
76. Szakács Z, Mészáros T, de Jonge MI, Gyurcsányi RE. Selective counting and sizing of single virus particles using fluorescent aptamer-based nanoparticle tracking analysis. Nanoscale. 2018;10(29):13942–8.
77. Rastogi M, Pandey N, Shukla A, Singh SK. SARS coronavirus 2: from genome to infectome. Respir Res. 2020;21(1):318.
78. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. 2024. Available online: https://covid19.who.int/
79. Ban DK, Bodily T, Karkisaval AG, Dong Y, Natani S, Ramanathan A, Ramil A, Srivastava S, Bandaru P, Glinsky G, Lal R. Rapid self-test of unprocessed viruses of SARS-CoV-2 and its variants in saliva by portable wireless graphene biosensor. Proc Natl Acad Sci USA. 2022;119(28):e2206521119.

80. Wu HB, Wang CH, Chung YD, Shan YS, Lin YJ, Tsai HP, Lee GB. Highly-specific aptamer targeting SARS-CoV-2 S1 protein screened on an automatic integrated microfluidic system for COVID-19 diagnosis. Anal Chim Acta. 2023;1274:341531.
81. Yi J, Xiao W, Li G, Wu P, He Y, Chen C, He Y, Ding P, Kai T. The research of aptamer biosensor technologies for detection of microorganism. Appl Microbiol Biotechnol. 2020;104(23):9877–90.
82. Nguyen TT, Kim ER, Gu MB. A new cognate aptamer pair-based sandwich-type electrochemical biosensor for sensitive detection of Staphylococcus aureus. Biosens Bioelectron. 2022;198:113835.
83. Wei L, Wang Z, Wang J, Wang X, Chen Y. Aptamer-based colorimetric detection of methicillin-resistant Staphylococcus aureus by using a CRISPR/Cas12a system and recombinase polymerase amplification. Anal Chim Acta. 2022;1230:340357.
84. Azmi UZM, Yusof NA, Abdullah J, Mohammad F, Ahmad SAA, Suraiya S, Raston NHA, Faudzi FNM, Khiste SK, Al-Lohedan HA. Aptasensor for the detection of Mycobacterium tuberculosis in Sputum Utilising CFP10-ESAT6 protein as a selective biomarker. Nanomaterials (Basel). 2021;11(9):2446.
85. Ye H, Duan N, Gu H, Wang H, Wang Z. Fluorometric determination of lipopolysaccharides via changes of the graphene oxide-enhanced fluorescence polarization caused by truncated aptamers. Mikrochim Acta. 2019;186(3):173.
86. Capatina D, Lupoi T, Feier B, Blidar A, Hosu O, Tertis M, Olah D, Cristea C, Oprean R. Label-Free Electrochemical Aptasensor for the detection of the 3-O-C12-HSL quorum-sensing molecule in Pseudomonas aeruginosa. Biosens (Basel). 2022;12(7):440.
87. Deb A, Gogoi M, Mandal TK, Sinha S, Pattader PSG. Specific instantaneous detection of Klebsiella pneumoniae for UTI diagnosis with a Plasmonic Gold Nanoparticle Conjugated Aptasensor. ACS Appl Bio Mater. 2023;6(8):3309–18.
88. Zivich PN, Grabenstein JD, Becker-Dreps SI, Weber DJ. Streptococcus pneumoniae outbreaks and implications for transmission and control: a systematic review. Pneumonia (Nathan). 2018;10:11.
89. Gingerich AD, Mousa JJ. Diverse mechanisms of protective anti-pneumococcal antibodies. Front Cell Infect Microbiol. 2022;12:824788.
90. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of Disease Study 2019. Lancet. 2022;400(10369):2221–48.
91. Carabalí-Isajar ML, Rodríguez-Bejarano OH, Amado T, Patarroyo MA, Izquierdo MA, Lutz JR, Ocampo M. Clinical manifestations and immune response to tuberculosis. World J Microbiol Biotechnol. 2023;39(8):206.
92. World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization; 2020.
93. Das R, Dhiman A, Mishra SK, Haldar S, Sharma N, Bansal A, Ahmad Y, Kumar A, Tyagi JS, Sharma TK. Structural switching electrochemical DNA aptasensor for the rapid diagnosis of tuberculous meningitis. Int J Nanomed. 2019;14:2103–13.
94. Li J, Hu K, Zhang Z, Teng X, Zhang X. Click DNA cycling in combination with gold nanoparticles loaded with quadruplex DNA motifs enable sensitive electrochemical quantitation of the tuberculosis-associated biomarker CFP-10 in sputum. Mikrochim Acta. 2019;186(9):662.
95. Moya C, Maicas S. Antimicrobial resistance in Klebsiella pneumoniae strains: mechanisms and outbreaks. Proceedings. 2020;66(1):11.
96. Chang D, Sharma L, Dela Cruz CS, Zhang D, Clinical, Epidemiology. Risk factors, and control strategies of Klebsiella pneumoniae infection. Front Microbiol. 2021;12:750662.
97. Song MY, Nguyen D, Hong SW, Kim BC. Broadly reactive aptamers targeting bacteria belonging to different genera using a sequential toggle cell-SELEX. Sci Rep. 2017;7:43641.
98. Kukushkin V, Ambartsumyan O, Subekin A, Astrakhantseva A, Gushchin V, Nikonova A, Dorofeeva A, Zverev V, Keshek A, Meshcheryakova N, Zaborova O, Gambaryan A, Zavyalova E. Multiplex lithographic SERS aptasensor for detection of several respiratory viruses in one pot. Int J Mol Sci. 2023;24(9):8081.
99. Effah CY, Ding L, Tan L, He S, Li X, Yuan H, Li Y, Liu S, Sun T, Wu Y. A SERS bioassay based on Vancomycin-modified PEI-interlayered nanocomposite and aptamer-functionalized SERS tags for synchronous detection of Acinetobacter baumannii and Klebsiella pneumoniae. Food Chem. 2023;423:136242.
100. Chang X, Zhang C, Lv C, Sun Y, Zhang M, Zhao Y, et al. Construction of a multiple-aptamer-based DNA logic device on live cell membranes via associative toehold activation for Accurate Cancer Cell Identification. J Am Chem Soc. 2019;141(32):12738–43.
101. Rozenblum GT, Lopez VG, Vitullo AD, Radrizzani M. Aptamers: current challenges and future prospects. Expert Opin Drug Discov. 2016;11(2):127–35.
102. Yu H, Zhu J, Shen G, Deng Y, Geng X, Wang L. Improving aptamer performance: key factors and strategies. Mikrochim Acta. 2023;190(7):255.
103. Ascione T, Barrack R, Benito N, Blevins K, Brause B, Cornu O, et al. General Assembly, diagnosis, Pathogen isolation - culture matters: Proceedings of International Consensus on Orthopedic infections. J Arthroplasty. 2019;34(2S):197–206.
104. Zhou Q, Natarajan B, Kannan P. Nanostructured biosensing platforms for the detection of food- and water-borne pathogenic Escherichia coli. Anal Bioanal Chem. 2023;415(16):3111–29.
105. Vidic J, Manzano M, Chang CM, Jaffrezic-Renault N. Advanced biosensors for detection of pathogens related to livestock and poultry. Vet Res. 2017;48(1):11.
106. Lehmann LE, Hauser S, Malinka T, Klaschik S, Weber SU, Schewe JC, et al. Rapid qualitative urinary tract infection pathogen identification by SeptiFast real-time PCR. PLoS ONE. 2011;6(2):e17146.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.