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REVIEW

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The application of aptamer in tuberculosis diagnosis: a systematic review

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Abstract

Tuberculosis represents a significant menace to health, leading to millions of cases and fatalities each year. Traditional diagnostic methods, while effective, have limitations, necessitating improved tools. Aptamers possessing remarkable specificity single-stranded DNA or RNA molecules promising in TB diagnosis due to their adaptability and precise biomarker detection capabilities. In this study, we aimed to evaluate the research on aptamer applications in TB diagnosis, evaluating the efficacy, limitations, and future prospects. The present systematic review study followed PRISMA guidelines, including peer-reviewed studies on aptamer efficacy in TB diagnosis. Eligibility criteria covered experimental and human studies on TB diagnosis, prognosis, progression, and treatment response. Of 1165 identified studies, 35 met inclusion criteria. Aptamers were utilized for MTB and mycobacterial antigen detection, showcasing notable sensitivity and specificity. Targeted antigens included ESAT-6, HspX, MPT 64, and IFN-γ. Various aptamer-based assays, such as electrochemical, fluorescent, and immunosensors, demonstrated effectiveness. Multiplex assays, particularly for IFN-γ, showed enhanced diagnostic accuracy. Aptamer-based assays exhibited discrimination between active TB and other conditions, showcasing their diagnostic value. Aptamers, especially in conjunction with nanomaterials, show promise in developing advanced TB biosensors with superior detection capabilities. Cost-effective devices with heightened sensitivity for clinical and screening use are crucial for TB control, emphasizing the need for ongoing research in this field.

Keywords Tuberculosis, Aptamers, Mycobacterium tuberculosis, Biosensors

Introduction

Tuberculosis (TB), caused by the bacterium Mycobacterium tuberculosis (MTB), presents a significant global health risk and leads to substantial illness and death on a worldwide scale [1]. This illness continues to be a major global health issue, with approximately 10 million new cases and 1.4 million deaths reported in 2019 alone [2]. In 2020, approximately 1.3 million fatalities worldwide were attributed to TB [3].

Prompt and precise recognizing tuberculosis is crucial for effectively managing, treating, and preventing disease transmission [4]. Studies have confirmed that conventional diagnostic techniques like sputum smear microscopy and culturing methods, often need to be improved by issues related to sensitivity, specificity, and turnaround time [5]. Bacterial examinations, such as acid-fast staining techniques in conjunction with mycobacterial cultures, continue to be the gold standard for diagnosing mycobacterial infections. The World Health Organization (WHO) recommends using rapid molecular tests as the primary diagnostic method for TB. However, their

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extensive implementation may require improvements due to their high cost in many laboratory settings [6–8]. Currently, in numerous developing nations, acid-fast staining microscopy of sputum samples continues to be extensively utilized for TB diagnosis because of its economic efficiency and quick results [9, 10]. In this case, the main challenge pertains to the unreliability and restriction of the sensitivity range, which fluctuates between 20% and 80% [11, 12]. According to the WHO, conventional acid-fast staining techniques only detect 28% of MTB cases as smear-positive [13].

Therefore, improved diagnostic tools are urgently needed for TB. In recent years, novel diagnostic technologies have offered promise in addressing these challenges [14]. Aptamers, which are individual strands of DNA or RNA possess remarkable strength and selectivity of binding for particular target molecules, are gaining growing interest for their potential use in diagnosing TB [15, 16]. The unique properties of aptamers, including their adaptability for use in various diagnostic platforms and their potential for rapid and precise detection of TB-related biomarkers, make them a promising target for investigation in the context of TB diagnostics [17].

Despite their potential, aptamers have not yet been widely adopted in clinical diagnostics, largely due to several key challenges. First, the selection process for aptamers, known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment), can be time-consuming and expensive, limiting large-scale development. Additionally, although aptamers are highly specific, their stability in biological environments remains a challenge, as they can be susceptible to degradation by nucleases [18]. Moreover, the standardization of aptamer-based diagnostic platforms and regulatory approval processes is still in its early stages, making their clinical application less straightforward compared to established diagnostic technologies [19].

Given the evolving landscape of TB diagnosis and the potential of aptamers as diagnostic tools, a comprehensive assessment of the current state of research on applying aptamers in TB diagnosis is warranted. Therefore, this systematic review aimed to consolidate existing evidence and critically evaluate the efficacy, limitations, and future prospects of aptamer-based technologies in diagnosing TB. By synthesizing the findings from relevant studies, this review aims to offer a thorough examination of the present state and prospective contributions of aptamers towards improving TB diagnosis, thereby contributing to advancing TB diagnostic strategies and the ultimate goal of TB control and eradication.

Methods

Eligibility criteria

We conducted the present systematic review systematic review registered with PROSPERO (ID: CRD42023484420), adhering to the PRISMA guidelines [20]. The inclusion criteria for the study included (A) any peer-reviewed research that examined the efficacy of aptamers in diagnosing tuberculosis (B) studies that investigated various aspects of TB diagnosis, prognosis, progression, and treatment response. The study had specific exclusion criteria that were applied to filter out research studies that had (A) incomplete or inadequate documentation, and (B) articles that were written in languages other than English. Also, studies that were presented in the form of letters to the editor, abstracts in conferences, review articles, case reports, and articles lacking required information were also excluded.

Information sources

We systematically explored articles from 1990 until November 2023 in databases such as the Web of Science (WOS), Scopus, PubMed, and Embase and Google Scholar. Additionally, we conducted searches in grey literature sources, specifically on allconferences.com, conferencealerts.com, opengrey, and oatd.org. Lastly, we examined the reference lists of the articles incorporated in our systematic review.

Search strategy

We used the MeSH (Tuberculosis, Aptamers, Infections and SELEX Aptamer Technique) and non-MeSH (Kochs Disease, Mycobacterium tuberculosis, Nucleotide and Aptamer-based technique) keywords to search related articles. This includes: #1 Tuberculosis or " Kochs Disease" or " Koch's Disease" or " Koch Disease" or " Mycobacterium tuberculosis Infection" or " Infection, Mycobacterium tuberculosis" or " Infections, Mycobacterium tuberculosis" or " Mycobacterium tuberculosis Infections"; and #2 "SELEX Aptamer Technique" or "Aptamers, Nucleotide" or Aptamer or "Aptamer-based technique".

Selection process

Two examiners (MR and EI) assessed the titles and abstracts of all identified studies to identify research relevant to this systematic review. In the subsequent phase, two researchers independently examined the complete texts of the studies to confirm their eligibility for inclusion based on the criteria outlined in Section "Eligibility Criteria." Any discrepancies were addressed through discussion, and in cases where disagreements could not be resolved, the third researcher (MHS) reached the ultimate decision (MHS). The third researcher reviewed the studies in question, considering both MR's and EI's

assessments. A predefined set of inclusion and exclusion criteria was applied to ensure objectivity in decision-making. Discrepancies were typically resolved by re-evaluating the study's methodology, relevance to the research question, and whether the study met the eligibility criteria. The third researcher's decision was considered final, and this process ensured that only studies that met all necessary criteria were included in the final review. The first and second phases of screening process was conducted utilizing the Endnote software version 9.

Data extraction

The process of collecting data involved individual extraction from the included articles by three researchers (MR, NF, and EI), adhering to the data extraction checklist. In cases where any disagreements remained unresolved, the final decision was made by the fourth researcher (MHS). The extracted data included the primary author's name, the country where the study was conducted, the year the article was published, the sample under investigation, the type of aptamer under investigation, and the results reported.

Results

Study selection

The steps of choosing articles is illustrated in Fig. 1 using the PRISMA flow diagram [20]. A sum of 1165 studies was obtained through searches in the databases and grey literatures (1093 records from the official databases and 72 records from the grey literatures). Prior to the initial screening phase, we excluded 412 records due to duplication. Two researchers initially screened the titles and abstracts of 681 articles. Subsequently, 621 articles (469 articles in one step and 152 articles in the next step) were excluded due to not meeting the inclusion and exclusion criteria. Out of the 60 studies chosen for a thorough examination of the full text, 25 studies were excluded based on the explanations provided in Fig. 1 and ultimately, this study included a total of 35 studies [4, 21–54].

Aptamers usage for M. Tuberculosis and mycobacterial antigens determination

Table 1 shows a summary of the studies done in this field. While there are various serological methods for identifying antibodies induced by Mtb, there is a specific period, referred to as a "window of time," during which mycobacterial antigens are present in serum samples without the concurrent production of host antibodies [55]. As a result, assessing the content of serum samples within this timeframe might yield a misleading negative result. Furthermore, existing serological methods, whether commercial or experimental, are incapable of discerning individuals with active TB from those who have received the BCG vaccine or are latently infected. In this scenario,

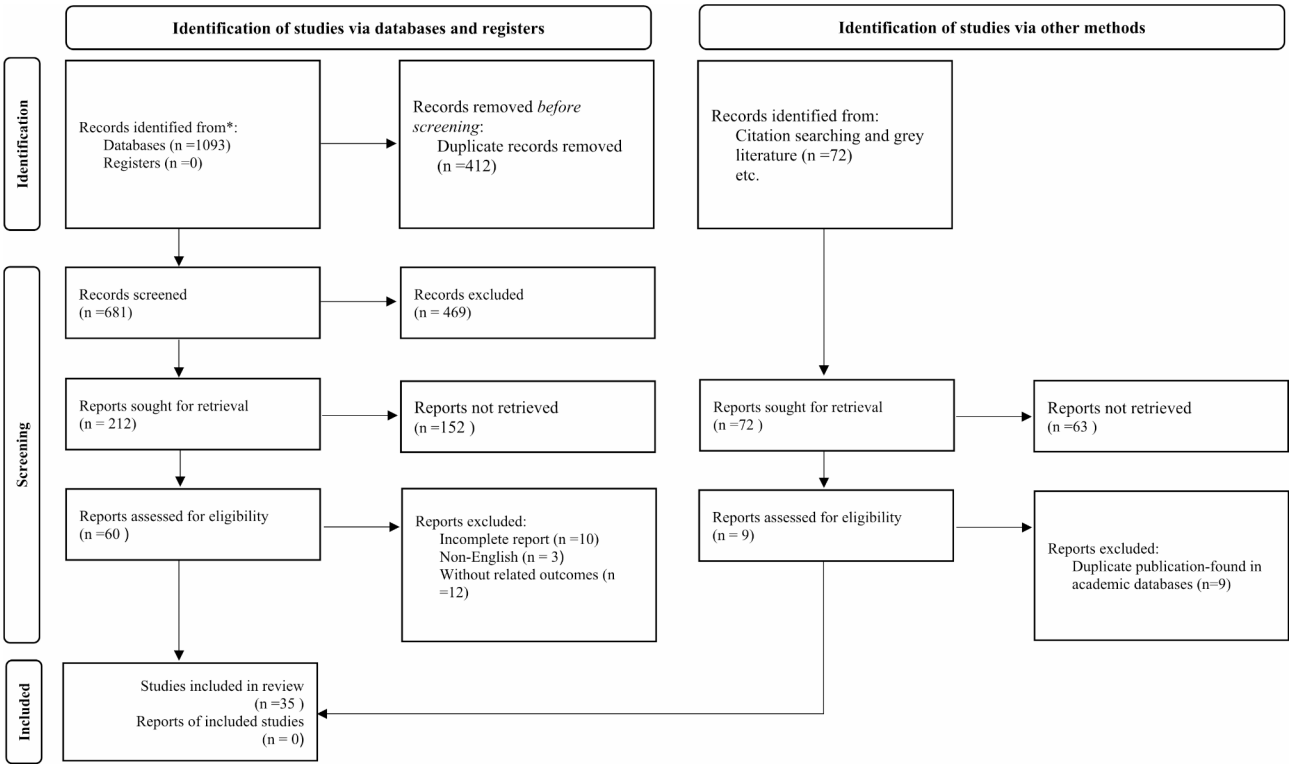

Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram (2020) of search process for studies examining the application of aptamer in Tuberculosis diagnosis: A systematic review

Table 1 The use of aptamers in the diagnosis of tuberculosis through the effect on antigens

First Author	Aptamer name	Target	Method	Approach for efficacy evaluation	Linear range	Aptamer sequency
Kil B (2023)	Single-stranded DNA aptamer	ESAT-6	Aptamer-based qPCR	LOD: 2.5 mg L^{-1}	NR	NR
Huang H (2023)	MXene/C60NPs/Au@Pt/APt2	ESAT-6	dual-signal output	LOD: 2.88 fg mL^{-1}	100 fg mL^{-1} to 50 ng mL^{-1}	NR
Bethu R (2023)	H63SL2-M6	HspX and MPT 64	ALISA technique	NR	NR	NR
Zhou Y (2021)	MPT64-A1	ManLAM	Aptamer-based immunohisto-chemistry (IHC) method	LOD: 2.5 mg L^{-1}	10–800 mg L^{-1}	NR
Azmi U (2021)	capture aptamer	CFP10-ESAT6 antigen	Electrochemical Aptamer-Based Assay	LOD: 2.5 ng/mL	5 to 500 ng/mL	50-NH2-GCC TGT TGT GAG CCT CCT AAC CCC ATC TTA TAC GTA TAT GGA CTC ATC TCG ACC CCC GAT AGG CTT GGT ACA TGC TTA TTC TTG TCT CCC-30
Kumari P (2019)	single stranded DNA	HspX	ALISA	NR	NR	'CTG ACGAGGCCATGCTAGATGCGAT3' and devR R spec 5'CCAGCGC CCAC ATCTTTGA3'
Li N (2019)	AuNPs/UiO-66-NH2	MPT64	Electrochemical measurements	< 10 fg·mL^{-1}	–8.1543–1.7625 pg·mL^{-1}	APT-I: 5'-SH-(CH2)6-TGGGAGCTG ATGTCGCATGG GTTTTGATCACATGA-3
Sypa-bekova M (2019)	single stranded DNA	MPT64	electrochemi-cal impedance spectroscopy	4.1 fm	0.1 fM to 5 nM	NR
Lavania S (2018)	H63SL2-M6	HspX	ALISA	NR	NR	NR
Zhang X (2017)	Single-stranded DNA aptamer	H37Rv	Immobilizing thiol-modified aptamer on an Au inter-digital electrode (Au-IDE)	100 cfu/mL	NR	5'-GGGAGCTCAGAATAAACGCT CAA (N35) TTCGACATGAGGCC CGGATC -3', forward primer 5'-GGGGAG CTCAGAATAAACGCTCAA-3'
Thakur H (2017)	Poly(3,4-ethylenedioxythio-phene) (PEDOT) doped with carbon nanotubes (CNTs)	MPT64	Electrochemical impedance	0.75 fg mL^{-1}		5'-[Btn]GTACAAACGACGGCCAG TCCTTGGGATGATTCAAGC AAAGCCTCACGCCTACGGCTA AGTC ATAGCTGTCTCTCCTG-3'
Sypa-bekova M (2017)	single stranded DNA	MPT64, ESAT-6 and CFP-10	sandwich enzyme linked oligo-nucleotide assay (ELONA)	k_d= 8.85 * 10^{-4}	NR	50- TCA CTT CAA ATG TGC GCT TC e N40 e CGT CAA AAC AGG GGG TAG AA – 30
Russell Th (2017)	SOMAmer	Mycolyl-transferase, antigen 85 A, FbpA	Agilent Technologies	LOD: 2.6 * 10^7 cells/ml	1.2 * 10^4 to 6.8 * 10^4 CFU/ml	NR
Bai L (2017)	GNPs-C60-PAn	MPT64 antigen	electrochemical impedance spectroscopy	20 fg/mL	0.02 to 1000 pg/mL	50-SH-(CH2)6-TGGGAGCTGATG TCGCATG GGTTTTGATCACATGA-3
Ansari N (2017)	Apt8 and Apt22	Ag85A (FbpA)	SELEX	Kd = 62.95 nM	2.1 nM	5'-GCTGTGTGACTCCTGCAAN43-GCAGCTGTATCTTGTCTCC-3'
Mozioglu E (2016)	Mtb36	Whole M. tuberculosis (H37Rv) cell	qPCR	Kd: 5.09 ± 1.43 nM	NR	NR

Table 1 (continued)

First Author	Aptamer name	Target	Method	Approach for efficacy evaluation	Linear range	Aptamer sequency
Tang X (2016)	T9	ManLAM	ELONA	Kd: 668 ± 159 nmol L^{-1}	NR	NR
Biag I (2015)	Mtb-Apt1 and Mtb-Apt6	AHAS	To inhibitory purposes	IC_{50}: 28.9 ± 0.002	NR	Mtb-Apt1: 5′CGAGTGAGGGCGAGGCGCG CTCCT GCCGGT-3′Mtb-Apt6:5′CGGCCA GGGGACGAGCGCGCCCTG ATCGTG-3′
Qin L (2014)	PAA1	Anti-MPT64 antibody	ELISA	Kd: 8.68 nmol L^{-1}	NR	NR
Tang X (2014)	CE15 and CE24	ESAT6 and CFP10	ELONA	Kd: 1.6×10^{-7} M	NR	NR
Rotherham L (2012)	CSIR 2.11	CFP-10. ESAT-6	ELONA	Kd: 861.07 nM	NR	NR

AHAS, Acetohydroxyacid synthase; ALISA, Aptamer Linked Immobilized Sorbent Assay ; ELONA, Enzyme-Linked Oligonucleotide Assay; ESAT-6, early secreted antigenic target 6; Qpcr, quantitative PCR ; LOD: Limit of Detection, ITC: Isothermal titration calorimetry; ManLAM, Mannose-capped lipoarabinomannan; NR, not reported, Kd: dissociation constant

the whole-bacterial selection method using aptamers is characterized as a straightforward, direct, and easily replicable approach that can be implemented without prior knowledge of the target molecules [56].

In an experimental investigation, Huang et al. assessed the performance of an electrochemical aptasensor utilizing dual-signal output for highly sensitive detection of MTB early secreted antigenic target 6 (ESAT-6) antigen. They found that using MXene/C60NPs/Au@Pt nanocomposite as signal amplification was able to detect the antigen successfully. As anticipated, the produced MXene/C60NPs/Au@Pt nanocomposite exhibited impressive redox activity and catalytic performance, leading to dual-signal output that effectively minimized background interference and enhanced sensitivity. Additionally, the suggested aptasensor demonstrated notable reproducibility, stability, and specificity [57, 58].

In identifying aptamer sequences influencing specific binding, Mozioglu and colleagues conclusively illustrated that exceptionally specific aptamers featured "G-repeats" at the 3' end of their structures and were enriched with "TGGGG," "GTGG," and "CTGG" motifs [36]. Numerous investigations have showcased the capability of aptamers to target crucial proteins and components of Mycobacterium tuberculosis, such as acetohydroxy acid synthase (AHAS) [45], mannose-capped lipoarabinomannan (ManLAM) [59], and polyphosphate kinase 2 [60]. ManLAM, the chief lipoglycan on the surface of M. tuberculosis, serves as an immunosuppressive epitope of the bacterium. Aptamer ZXL1 was created to specifically attach to ManLAM originating from the virulent Mtb strain H37Rv [61]. Hence, the aptamer ZXL1 has the potential to function as an innovative antimycobacterial agent and as an immune adjuvant for tuberculosis vaccines.

Other antigens that were evaluated as targets in the aptamer-based assay are heat shock protein X (HspX) antigen and MPT 64. The 16-kDa HspX antigen is crucial for the persistence of Mycobacterium tuberculosis under harsh conditions and facilitates the bacterium's replication. The MPT-64 antigen, or Rv1980c, serves as a particular virulence factor and is generated and released exclusively by actively dividing cells. The outcomes of their investigation indicated that the aptamer-linked immobilized sorbent assay (ALISA) technique for mycobacterium antigens HspX and MPT 64 proved to be a swift and cost-effective test (costing 1–3 dollars per test) with high sensitivity and specificity, comparable to existing methods [62, 63]. An aptamer known as H63SL2-M6, designed to target HspX, was proven effective in detecting HspX in the sputum of individuals with TB. This detection was achieved through the ALISA method, and the performance of ALISA surpassed that of traditional ELISA [27].

During infection, the natural immune system responses, characterized by the secretion of antibodies, can be employed as diagnostic and prognostic signals, providing insights into the presence of pathogens at different infection levels. Nevertheless, variations in antigenic properties occur due to the acquisition of recombinant proteins from diverse batch processing or the influence of contaminants originating from cloning vectors. This discrepancy leads to fluctuations in assay accuracy [64, 65]. Hence, Zhu et al. designed a diagnostic approach for pulmonary tuberculosis that employs biotinylated aptamers targeting anti-MPT64 antibodies. They showed that the ELISA method achieved a minimum

detection limit of 2.5 mg/L. Also, it has been reported a considerable sensitivity (64%) and specificity (94%) for this method [40]. Comparable results were documented in other study by Sypabekova et al. Their investigation findings demonstrated that using a ssDNA aptamer designed to identify the MPT64 protein for tuberculosis diagnosis specifically exhibited notable sensitivity and specificity [24]. One of the limitations of this method in some studies has been its low detection limit. For solving this problem, in some studies researchers used from nanohybrid structures by synthesizing fullerene-doped polyaniline (C60-Pan) and decorating them with MPT64-specific aptamers. As a consequence, an extraordinarily sensitive electrochemical aptasensor was developed, boasting a limit of detection (LOD) of 20 fg mL^{-1} [32].

In other studies, scientists employed a portable electrochemical biosensor based on aptamer-antibody sandwiches to detect tuberculosis-associated antigens. Azmi et al. evaluated the efficacy of this method against CFP10-ESAT6 antigen and they found a substantial correlation with the culture method, demonstrating 100% efficacy [22]. Proteins known as Culture Filtrate Protein-10 (CFP-10) and ESAT-6 are released upon TB infection. The aptamer CSIR2.11 can identify both the CFP-10-ESAT-6 complex and CFP-10 alone in sputum samples obtained from individuals with TB. In sputum samples from TB patients, CSIR 2.11 demonstrated a sensitivity of 100% and a specificity of 68.75% [41]. Moreover, a point-of-care (POC) diagnostic electrochemical sensor (ECS) device was created using the H63SL2-M6 aptamer for rapid diagnosis [27]. The CFP10-ESAT6 complex, exclusively released by M. tuberculosis during its initial cultivation period, exhibited increased sensitivity in identifying the presence of Mtb in compared to individual components [66]. These two distinct biomarkers for tuberculosis are absent in numerous non-tuberculous mycobacteria and the M. bovis BCG vaccine variant [55].

Table 2 Various categories of aptamers employed in biosensing methods related to IFN-γ in TB detection

First Author	Method name	Aptamer type	Efficacy amounts	Linear range
Taghdisi S	amplified fluorescent aptasensor	Single-stranded DNA aptamer	LOD: 10 pM	0.01 to 100 nM
Wang X	Immunosensor	Single-stranded DNA aptamer	LOD: 1.6 pg/mL	1.5–200 pg/mL
Tuleuova N	surface plasmon resonance	Single-stranded DNA aptamer	35 pM	0.2 to 333 nM
Parate K	Electrochemical immunosensor	capture aptamer	100–5000 pg/mL	25 pg/mL
Yao X	Microfluidic biosensor	T-DNA/MCH/CP/AuNCs-Gr@ZIF-8/GCE	11.43 pg/mL	10–10,000 pg/mL
Kim H	Liquid crystal aptasensor	APTES/DMOAP-treated glass-aptamer-ethanolamine	17 pg/mL	0.0169–844 pg/mL

Aptamers based on interferon gamma detection

The drive to create innovative technologies for detecting cytokines, particularly when the immune system encounters antigens from mycobacteria, is growing more compelling. In response to antigen stimulation, IFN-γ is primarily produced by macrophages and T helper cells (CD4+) [67]. This production is utilized to ascertain past exposure to infectious diseases. Clinically, the detection of IFN-γ release by T-cells is an indication of severe TB. In experimental models, it is utilized to validate the presence of immune T cells associated with the disease. IFN-γ's primary function is to regulate the immune reaction at a cellular level to both viral and TB agents [68]. The Interferon-gamma release assay (IGRA) is a blood test based on the immune system's response to detect MTB infection. The test measures T cell immune response to TB antigens, producing interferon-gamma upon re-exposure. A remarkable interferon-gamma production is presumed to indicate a tuberculosis infection [69]. A limitation of the IGRA test is its reduced sensitivity in detecting active TB, particularly in individuals with weakened immune systems. The assay cannot distinguish between active and latent tuberculosis infections, rendering it inappropriate for diagnosing active TB disease [70]. Therefore, according to the limitations of these conventional assay techniques, using aptamers as a new method has been considered in new studies.

Table 2 summarizes various categories of aptamers employed in biosensing methods related to IFN-γ. Different research teams have documented specific aptamers targeting or participating in pathways associated with IFN-γ. Liu et al. in an experimental study reported the description of a biosensor based on a Methylene Blue (MB) redox-tagged DNA hairpin aptamer for the identifying of IFN-γ [71]. Some studies have used the fluorescent aptasensor. Within fluorescent sensors, the target molecule or biorecognition element is marked with a fluorescent label, and the fluorescence intensity indicates the strength of the interaction between the biorecognition molecule and the target. Zhang and colleagues designed a fluorescence biosensor for detecting IFN-γ with exceptional sensitivity and specificity, utilizing a

G quadruplex aptamer bonded with malachite green (MG). They attained a detection limit of 7.65 fM in a buffer, exhibiting a linear range from 0 fM to 20 pM and a recovery range of 95.29–118.08% in real samples. Their achievement reflects a notable sensitivity and specificity toward the target [72]. In the research conducted by Wen et al., they also created a fluorescent sensor, but their approach involved DNA click polymerization. They devised a novel fluorescent sandwich structure consisting of aptamer/protein/aptamer. This configuration demonstrated sensitivity, selectivity, and stability in detection [73].

In recent years, a novel electrochemical aptasensor has been developed for evaluating IFN-γ, utilizing a modified electrode with graphene and gold nanoparticles (AuNPs) and incorporating dual enzyme-assisted signal amplification. This particular sensor has achieved an impressively detection threshold as low as 2 picomolar (pM) and demonstrated a broad linear detection range from 5 pM to 5 nanomolar (nM). When IFN-γ was present, binding of the aptamer to IFN-γ resulted in the detachment of aptamers from the electrode surface. Concurrently, RecJf exonuclease digested the aptamers, making IFN-γ accessible for target recycling. In this procedure, IFN-γ was utilized as capture probes, which interacted with linker probes and reporter probes labeled with biotin to form hybridizations [48].

If the biorecognition component of the biosensor is an antibody, it is classified as an immunosensor. Immunosensors exploit the high affinity between antibodies and antigens to detect particular analytes by employing an appropriate signal transducer. Sanchez-Tirado and collaborators engineered an electrochemical immunosensor designed for the detection of IFN-γ in saliva. The biosensor utilized a sandwich-type immunoassay, involving the attachment of a capture anti-IFN-γ antibody to the electrode surface through the diazonium salt grafting of p-aminobenzoic acid. They attained a detection threshold of 1.6 picograms per milliliter (pg/mL) and a linear range from 2.5 to 2000 pg/mL [74].

Furthermore, Ruecha and colleagues devised a label-free impedance immunosensor constructed on paper. They augmented the paper-based electrode with polyaniline-graphene and immobilized human IFN-γ antibody onto it. Electrochemical impedance was employed for the specific detection of interferon-gamma in human serum, showcasing a sensitive, straightforward, fast, and cost-effective method. The achieved results include a detection limit of 3.4 picograms per milliliter (pg/mL), a linear range of 5–1000 pg/mL, and a recovery range of 101–104% in human serum [49]. Zhang and colleagues created a sandwich-type electrochemical immunosensor. They devised a disposable indium tin oxide electrode to construct their immunosensor. The immunosensor they developed demonstrated a detection threshold of 0.048 picograms per milliliter (pg/mL) along with an extensive linear range spanning from 0.1 to 1×104 pg/mL [50].

Recently, microfluidic devices have surfaced as potential diagnostic tools suitable for deployment in resource-limited countries. These chip-based sensing techniques utilize minimal sample volumes, enabling real-time, point-of-care diagnosis for infectious diseases. Microfluidics facilitates the analysis of various clinical samples, including urine, blood, or saliva [75].

In recent years, there has been a notable focus on multiplex assay detection methods. Multiplex assay detection enables the simultaneous measurement of multiple analytes [76]. Wang and colleagues proposed that employing multiplex biomarker assays, rather than single cytokine assays, for diagnosing active TB could improve diagnostic accuracy. They utilized a microbead-based multiplex assay to analyze several biomarkers, and from these, five chemokines/cytokines were identified as capable of distinguishing between patients with active pulmonary TB and healthy controls. They found that utilizing a multiplex approach significantly enhanced the diagnostic performance for TB when compared to individual detection methods [77].

Discussion

The systematic review presented in this manuscript highlights the growing interest in aptamer-based technologies for the diagnosis of TB, specifically focusing on their ability to detect MTb antigens with high sensitivity and specificity. Given the limitations of traditional TB diagnostic methods, including sputum smear microscopy and culture, aptamer-based assays offer an innovative and promising alternative, particularly in settings where rapid and accurate diagnosis is critical.

The studies reviewed encompassed a diverse range of aptamer applications, focusing on the detection of MTB and mycobacterial antigens [38]. Notably, the aptamer-based assays exhibited promising results regarding sensitivity, specificity, and cost-effectiveness. Various studies utilized different aptamer sequences targeting specific MTB components, such as ESAT-6, HspX, and MPT 64. The aptamer-based assays demonstrated the capability to distinguish between active TB and alternative conditions, showcasing their potential as valuable tools in TB diagnosis [26, 27, 62]. In the realm of IFN-γ detection, a key marker for TB, aptamer-based biosensors showed considerable promise. These biosensors, whether employing electrochemical, fluorescent, or immunosensor approaches, demonstrated elevated levels of accuracy and selectivity. The versatility of aptamers was evident in their ability to target IFN-γ and contribute to developing innovative diagnostic technologies [48, 78].

ESAT-6 is one of the most extensively studied TB antigens, particularly due to its absence in the BCG vaccine strain and non-tuberculous mycobacteria, making it a specific marker for active M. tuberculosis infection [79]. These aptamer-based platforms offer a significant advantage in distinguishing between active TB and latent TB or BCG-vaccinated individuals, addressing one of the major limitations of traditional diagnostic methods. The ability to detect such low antigen levels suggests that aptamers targeting ESAT-6 could play a crucial role in early diagnosis, helping to curb TB transmission and improve patient outcomes [80].

HspX is another antigen critical for the survival of M. tuberculosis under stress conditions, particularly during latent infections. Aptamer-based assays targeting HspX, such as the H63SL2-M6 aptamer, have demonstrated the capability to detect HspX in clinical samples like sputum [81]. The stability and high binding affinity of these aptamers make them particularly suitable for use in resource-limited settings, where robust and reliable diagnostic tools are required. The high sensitivity of aptamer-linked assays for HspX highlights their potential as diagnostic tools for detecting both active and latent TB infections, especially in cases where traditional methods may fall short [82].

MPT64 is another antigen secreted by actively dividing M. tuberculosis cells and has been the target of numerous aptamer-based diagnostics [83]. Studies have shown that MPT64-specific aptamers can be used in various platforms, including electrochemical biosensors and ELISA-like assays, to achieve highly sensitive detection [84]. Some studies have shown that aptamer-based sensors for MPT64 can detect antigen concentrations at femtogram levels, indicating their utility in diagnosing early-stage TB [85, 86]. These biosensors not only offer high sensitivity but also provide a rapid and cost-effective alternative to traditional TB diagnostics, making them highly suitable for widespread use in TB-endemic areas [86].

Moreover, aptamers targeting these antigens, particularly when combined in multiplex assays, offer a significant advantage by allowing simultaneous detection of multiple TB biomarkers. This multiplexing capability enhances the overall diagnostic accuracy, especially in differentiating between active TB, latent infections, and other pulmonary diseases that may present similar clinical symptoms [19].

Aptamer biosensors represent a cutting-edge tool in TB diagnostics, offering unique advantages in sensitivity and specificity, especially in cases where traditional diagnostic methods struggle. In paucibacillary TB, where bacterial load is low, conventional methods like sputum smear microscopy often yield false negatives due to insufficient bacteria in clinical samples [87]. Aptamer-based biosensors, with their ability to detect extremely low concentrations of antigens, provide a significant advantage in these cases. Studies have shown that aptamers can detect key antigens such as ESAT-6, HspX, and MPT64 at femtogram levels, making them highly suitable for diagnosing TB even in paucibacillary conditions, where bacterial presence may be minimal. This capability could be particularly beneficial in early diagnosis or in patients with low bacterial loads [17].

For extra-pulmonary TB, where traditional methods like sputum tests are less effective due to the non-respiratory nature of the infection, aptamer biosensors offer a promising alternative. By targeting specific TB biomarkers present in blood, cerebrospinal fluid, or tissue samples, aptamers can provide non-invasive diagnostic options for detecting TB in locations outside the lungs. For instance, biosensors targeting antigens such as MPT64 or ManLAM have shown promise in detecting extra-pulmonary TB with high sensitivity [18]. This ability to detect TB across a variety of sample types significantly broadens the applicability of aptamers in TB diagnostics, addressing a crucial gap in current testing methods [88].

In addition to diagnosing paucibacillary and extra-pulmonary TB, aptamers also hold potential in the detection of drug-resistant TB. Aptamers can be engineered to specifically bind to mutated forms of Mycobacterium tuberculosis proteins associated with drug resistance. For example, aptamers have been designed to target rpoB gene mutations, which are linked to rifampicin resistance, one of the key markers of multidrug-resistant TB (MDR-TB) [12]. Aptamer-based biosensors could therefore serve as a rapid and cost-effective means of identifying drug-resistant strains of M. tuberculosis, enabling faster treatment decisions and helping to curb the spread of resistant TB strains [89].

In summary, aptamer biosensors provide several key benefits in diagnosing paucibacillary and extra-pulmonary TB, and they also show promise in identifying drug-resistant TB strains. These capabilities further highlight the versatility and potential of aptamer-based diagnostics in addressing some of the most challenging aspects of TB detection and management.

The study conducted was characterized by various advantages. These include a thorough examination of the current level of research on applying aptamers in tuberculosis diagnosis, well-defined eligibility criteria, a strict selection process, and comprehensive data extraction.

It's crucial to emphasize that the present study has a few limitations that may impact the results:

1. The findings may not be generalizable due to variations in study populations, methodologies, and aptamer applications across different studies.

2. Despite a detailed extraction process, some studies may require more comprehensive documentation or sufficient data, limiting the depth of analysis.

Conclusions and future perspectives

In nations where TB prevalence is high, achieving affordable and dependable diagnostic tools is a key objective for disease control. Biosensors, particularly aptasensors, are positioned as crucial components in addressing this need. This review emphasized a diverse range of aptasensors specifically crafted to identify TB, demonstrating a detection sensitivity within the femtomolar range for biomolecules linked to the pathogen. Given the crucial role of diagnosis during the latent phase, there remains a necessity to create cost-effective devices with reduced detection thresholds suitable for clinical and screening applications.

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EI, MHS, and MR designed the study and analyzed the data. MR, EI and NF cooperated in the implementation of the study. NF conceived the study, and lead study design, data collection, supervision, validation, proofreading and approval of the study.

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No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The present work is approved by the ethical committee of North Khorasan University of Medical Sciences. The ethical approval Code is IR.NKUMS.REC.1403.082.

Consent for publication
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The authors declare no competing interests.

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