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REVIEW ARTICLE **OPEN ACCESS**

Gene Editing for Tuberculosis using CRISPR-Cas Technology

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Abstract:
Advances in genome editing technologies are simplifying the tedious, laborious work in research. Recent advancements have introduced innovative genome editing techniques, such as the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and CRISPR-associated protein (Cas) system, which have proven to be powerful tools in genome editing. Unlike previous methods, CRISPR-Cas is highly specific and capable of gene knock-in, knock-out, or knockdown, which aids researchers. Such a tool is valuable for diagnosis, treatment, and vaccine production against life-threatening, mutating, and drug-resistant diseases such as Tuberculosis (TB).

Mycobacterium Tuberculosis (Mtb) and other members of the Mycobacterium Tuberculosis Complex (MTBC) are notorious and slow-growing bacteria; hence, they are difficult to tackle. Different CRISPR-Cas systems, for example, Cas9, Cas12a, Cas12b, and Cas13a, are the types being used in the diagnosis of Mycobacterium tuberculosis.

We have attempted to provide an overview of CRISPR-Cas technology and its application in tuberculosis. The literature was searched across platforms like PubMed, Google Scholar, Web of Science, SciELO, and Scopus. The keywords that were used to search the related literature were 'Mycobacterium tuberculosis OR Tuberculosis,' 'TB AND Diagnosis,' 'TB Vaccines AND Treatment,' 'Mycobacterium tuberculosis AND CRISPR-Cas,' 'CRISPR-Cas system,' 'CRISPR-Cas in TB diagnosis,' 'Cas9,' 'Cas12a,' 'Cas13,' 'gene editing mycobacteria,' and 'CRISPRi Mycobacterium tuberculosis.' The approach to searching for and selecting articles for the review provided a comprehensive and well-supported analysis of the subject matter.

The review emphasizes the novel technology CRISPR-Cas system for diagnosis, treatment, and the development of new TB vaccines. Along with the applications, the review comprises traditional methods of diagnosis of TB and mechanisms and challenges related to CRISPR-Cas. Using the CRISPRi technology, new targets were found as promising novel drug targets. Vaccine development with the help of this tool is also mentioned in this review.

CRISPR-Cas technology has emerged as a powerful tool for advancing TB research. Cas9, Cas12a, Cas12b, and Cas13 have demonstrated high specificity and sensitivity in detecting Mtb, even in samples with low bacterial loads. Moreover, CRISPR-mediated gene silencing has enhanced our understanding of the virulence mechanisms of the bacterium, facilitating the identification of novel therapeutic targets for designing new anti-TB drugs.

Tuberculosis continues to pose a major global health challenge due to the persistence of the pathogen and the emergence of drug-resistant strains. CRISPR-Cas technology offers innovative solutions, and its applications extend beyond diagnostics to include drug discovery, therapeutic development, and vaccine research. Thus, CRISPR-Cas systems can significantly accelerate efforts toward effective control and eradication of tuberculosis.

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1. INTRODUCTION

Mycobacterium Tuberculosis (*Mtb*) is a member of the MTBC family and is among the most notorious bacteria causing TB. TB is an airborne infection that mainly affects the lungs and results in Pulmonary Tuberculosis (PTB). Apart from PTB, this bacterium is linked to several human illnesses, including metabolic syndromes, immunological disorders, and pulmonary problems, according to mounting data [1]. According to the World Health Organization (WHO) Report 2024, globally, 10.8 million cases of TB were documented, of which 8.2 million cases were newly diagnosed with TB [2]. Such increasing cases of TB pose a threat to global health. The issue is further aggravated by the rise in incidents of antimicrobial resistance to over-the-counter antibiotics [2]. It is therefore necessary to eliminate or manage this infection with the aid of developing technologies. Historically, TB was diagnosed based on patient symptoms such as hemoptysis (blood in the cough). In 1890, at his Berlin conference, Robert Koch proposed tuberculin as a medication for the disease, though it was later rejected as a treatment and instead suggested for diagnosis [3]. Later, in 1908, Charles Mantoux created an intradermal injection of tuberculin using a syringe and cannulated needle. In the 1930s, Florence Seibert of the Phipps Institute at the University of Pennsylvania produced a Pure Protein Derivative [PPD] in its present form after conducting several investigations [4]. As studies into the disease increased, it was diagnosed using various techniques, including staining [Ziehl-Neelsen Carbol Fuchsin], culture methods [Lowenstein-Jensen solid media], and X-ray examination of cavitary lesions. Increased knowledge of tuberculosis led to the introduction of immunological techniques for diagnosis, such as the enzyme-linked immunosorbent spot assay for cells that produce interferon-gamma [5]. At present, with the tests mentioned above, WHO also recommends tests like the molecular line probe assay and GeneXpert, which are valuable for detecting specific genetic markers associated with drug resistance, particularly in cases of MDR-TB or XDR-TB. With increasing technological advances, Whole Genome Sequencing [WGS] has also become a choice for TB diagnosis [6].

Once the disease has been diagnosed, medication is required to treat it. Before streptomycin was first developed as a therapy, earlier therapies for this illness, such as the royal touch and the sanatorium cure, were practiced [7]. The introduction of streptomycin altered the therapy paradigm, followed by rifampicin and isoniazid [8, 9]. With the discovery of new drugs and their combination, the fatality rate has decreased. According to the recommendation of the WHO, the initial treatment includes the first-line drugs, such as Isoniazid [INH], Rifampicin [RIF], Pyrazinamide [PZA], and Ethambutol [EMB], for 6 months. In the case of Multidrug-Resistant-TB [MDR-TB] or Rifampicin-Resistant-TB [RR-TB], the patient is treated with the second-line injectable drugs such as Fluoroquinolones [Ofloxacin [Ofl], Levofloxacin [LVX], and Moxifloxacin [MFX]] and Aminoglycosides [Amikacin [Am], Kanamycin [Kan], and Capreomycin [Cap]]. New therapy for MDR/RR-TB, known as BPaLM, consists of bedaquiline [B], pretomanid [Pa], linezolid [L], and moxifloxacin [M] for 6 months, and for those with pre-XDR-TB, the regimen can be taken without moxifloxacin [BPaL] for 9 months. [6]. Though the WHO recommends a variety of antibiotic regimens, drug-resistant TB treatment is a clinical conundrum. Therefore, it is imperative to find newer avenues and targets for intervention and mitigation.

Current scientific discoveries have opened new possibilities for diagnosing and treating tuberculosis. The CRISPR array, previously only employed as a phylogenetic marker in TB, now uses different Cas systems for diagnosis, new treatments, and enhancement of TB research [10]. The CRISPR-Cas system, which is a component of the immune system of prokaryotic cells, guards them against foreign genetic material. This system consists of two major components: the CRISPR locus, which is transcribed and subsequently cleaved to produce short CRISPR RNAs [crRNAs], and the Cas proteins, which are encoded alongside [11]. The CRISPR locus has spacer sections that contain the foreign genetic material that must be cleaved, as well as palindromic repeats after each spacer sequence, whereas Cas proteins are a kind of nuclease that cleaves the foreign nucleotide sequences at certain points [12]. This review focuses on the detection and novel drug development to eliminate TB. The review will also discuss the challenges concerning CRISPR-Cas technology in diagnosis and treatment.

2. METHODOLOGY

This literature review aims to compile existing knowledge, highlighting current research and advancements in using CRISPR-Cas9 technology for diagnosing and treating tuberculosis. The review involved searching for relevant articles on various academic databases like PubMed [https://pubmed.ncbi.nlm.nih.gov/], Google Scholar [https://scholar.google.com/], SciELO [https://www.scielo.org/en], Web of Science [https://mjl.clarivate.com/home], and Scopus [https://www.scopus.com/sources.uri?zone=TopNavBar&origin=searchbasic] using specific search terms like '*Mycobacterium tuberculosis* OR Tuberculosis', 'TB AND Diagnosis', 'TB Vaccines AND Treatment', 'Mycobacterium *tuberculosis* AND CRISPR-Cas', 'CRISPR-Cas system', and 'CRISPR-Cas in TB diagnosis', 'Cas9', 'Cas12a', 'Cas13', 'gene editing *mycobacteria*', and 'CRISPRi *Mycobacterium tuberculosis*'. Additionally, reference lists from the identified articles and reviews were checked, and a forward and backward citation search was conducted to find additional relevant papers. No date restrictions were applied to the search, and all the relevant data were extracted. While extracting the peer reviews, articles, and reports, only the papers with the mentioned keywords were analyzed, whereas non-English papers, non-TB pathogens, and preprints were excluded in this review. High-impact papers were analyzed and thoroughly reviewed.

3. TRADITIONAL METHODS OF DIAGNOSIS OF TB DISEASE

Before diagnosis, it is necessary to understand how a person gets infected. Once the person gets infected with TB, they start showing signs and symptoms related to it, but in some cases, it is asymptomatic. As *Mtb* can infect any part of the body, the signs and symptoms depend on the site of the infection. In general, symptoms observed include fever, night sweats, and weight loss [13]. Understanding the symptoms and signs is important. The gold-standard diagnostic tool for detection is radiological examination [*e.g.*, X-ray], which is preceded by microbiological investigations [14, 15]. Mantoux Tuberculin Skin Test [TST], Interferon γ Release Assay [IGRA], and, when indicated, Nucleic Acid Amplification [NAA] testing of sputum are done to confirm the disease [14–17].

3.1. Radiological Examination

The chest X-ray of a TB patient may show a caseous mass in the lungs. To further confirm the diagnosis, other tests are done to avoid misdiagnosis. Cases like Solitary Fibrous Tumours of the Pleura [SFTP] show the same ball-like structure as in TB, which is a common misinterpretation. Hence, radiological examination is not specific to tuberculosis [18].

3.2. Microbiological Investigations

The microbiological investigation includes culturing and staining processes where the TB bacteria are grown and observed. These bacteria grow slowly and require 6-8 weeks. Other bacteria in the sample grow much faster and contaminate the culture. Due to this, Ziehl-Neelsen staining becomes challenging. Even if the staining is done successfully, it does not indicate anti-TB drug resistance. Hence, it becomes difficult to detect TB when the number of bacteria in the sample is low, whereas culturing it becomes strenuous. Therefore, culturing TB bacteria is time-consuming and significantly prolongs the diagnosis [19].

3.3. Mantoux Tuberculin Skin Test [TST]

The TST is the most widely used, given its feasibility and low cost. The TST is performed on the forearm of the person suspected of TB. A PPD [tuberculin] is injected in the forearm, producing an elevated skin reaction, *i.e.* wheal. After injecting the tuberculin, the area is measured at 48-72 hours. If the person has TB, the area shows inflammation. The disadvantage related to this test is that it cannot differentiate between latent infection and active TB and fails to diagnose intraocular TB [20]. This test also yields false-positive results with BCG vaccination, which is a problem for newly vaccinated infants [19]. It fails to detect the disease in immunosuppressed and extrapulmonary patients [21].

3.4. Interferon Gamma Release Assay [IGRA]

The IGRA test is more specific than the TST but has its fair share of disadvantages. Similar to TST, this test also fails to distinguish between latent and current infection. It does not show any evidence of infection in immunosuppressed individuals [20].

3.5. Nucleic Acid Amplification Test [NAAT]

According to the WHO, nucleic acid amplification tests are highly recommended and have overcome some drawbacks of previous tests. This test includes Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat, Genotype MTBDRplus, and Genotype MTBDRsl [2].

Xpert MTB/RIF and Xpert MTB/RIF Ultra: These tests are cartridge-based and include reagents and primers for *Mtb* resistance genes. The sample is liquefied before being placed into the cartridge. After loading, the entire cartridge is inserted into the machine [22]. These samples are detected for 81 core *rpoB* gene panels, including IS6110 and IS1081 for *Mtb* detection. Various versions, such as Xpert XDR, can identify types of *Mtb* drug resistance [17]. As with all other tests, this test also has certain limitations, such as the need for costly maintenance and electricity, and it shows no resistance in the case of poly-resistant TB [23]. Along with these limitations, it has suboptimal sensitivity and a high rate of false positive results, and it requires experience in handling samples [24]. Another issue is that cartridge shelf life is only 18 months, which makes it difficult for low-income nations to manage the cost [25].

Truenat: A chip-based, real-time PCR platform for TB diagnosis that is a portable and battery-operated machine. In this test, the sample is liquefied, and DNA extraction is performed. The extracted DNA is then mixed with the reagents of Truenat and loaded onto the chip, and real-time results are observed. Other versions of this test are Truenat MTB, Truenat MTB Plus, and Truenat MTB-Rif-Dx for the detection of *Mtb* as well as resistance genes [26]. The issue related to this test is that any mutation that occurs within the resistant gene is not detected through this test. Another disadvantage of this test is the viability of the bacilli, which can hinder the treatment process.

Genotype MTBDRplus and Genotype MTBDRsl: The test consists of three important steps, including DNA extraction, multiplex PCR amplification, and reverse hybridization. Reverse hybridization is performed on a special strip that contains zones where hybridization occurs. The associated disadvantages include a low bacillary count and the need for appropriate expertise for the test [27]. In a nutshell, all the above test shortcomings, viz., sensitivity, specificity, high maintenance, cost, expertise, bacillary count, and appropriate space, are the most important criteria to carry out the test and get appropriate results.

4. CRISPR-Cas TECHNOLOGY

The CRISPR-Cas system protects bacteria and archaea against invading conjugative plasmids, transposable elements, and viruses [28]. Jennifer Doudna and Emmanuelle Charpentier, with their discovery related to the CRISPR-Cas9 system as a gene editing tool, made genetic editing more precise and efficient [28]. As

depicted in Table **1**, there are two classes of the CRISPR-Cas system, *i.e.*, Class 1 and Class 2, and each class is further divided into different types and subtypes [29],[30]. *Mycobacterium tuberculosis* contains a Class 1, type III-A CRISPR-Cas10 system as its defence system that targets foreign nucleic acids of plasmids as well as phages [31–33]. This type exhibits three enzymatic activities to cleave foreign nucleic acids [34–36].

4.1. Mechanism of the CRISPR-Cas System

The CRISPR-Cas system in prokaryotes operates through three stages: adaptation, crRNA biogenesis/expression, and target interference, shown in Fig. (**1**) [37].

[i] Adaptation: When a prokaryotic cell encounters foreign genetic material, such as a virus or plasmid, a small segment of it is cut and integrated into the CRISPR array in the spacer region called a protospacer. This process is facilitated by the Cas1 and Cas2 proteins, which are present in most CRISPR classes, except for type III C, D, and type IV [37, 38]. Although some research articles advocate a Reverse Transcriptase [RT]-fused-Cas1 protein in the type III-B system of *Marinomonas mediterranea* [39], in 2022, Aviram *et al.*'s study on the type III-A CRISPR-Cas system revealed that the adaptation system is RT-free [40]. More research is needed on the Class 1 CRISPR-Cas system to study its adaptation system. After the cleavage of foreign genetic material that integrates into the CRISPR array, the prokaryotic cell is allowed to "memorize" the invader's genetic information. It enables the bacterial immune system to recognize and mount a response against the same invader if it reappears. Some CRISPR types may use ancillary proteins to aid in the adaptation process [41].

[ii] crRNA biogenesis/expression: The CRISPR array is transcribed into a precursor RNA transcript [pre-crRNA] and contains the spacers derived from the foreign genetic material. This precursor transcript is further processed to produce mature CRISPR RNAs [crRNAs] that carry the specific sequence information of the invader. The maturation of pre-crRNA into mature crRNA is mediated by specific proteins. For example, in class 1 CRISPR systems, the cas6 gene plays a major role in this process, while in type II systems, cas6 is absent and RNase III takes on the role. In type V systems, the *cpf1* gene is involved in both adaptation and crRNA biogenesis/expression [41].

[iii] Target interference: The mature crRNA serves as a guide to locate the specific genetic material of the invading element. Class 1 CRISPR systems utilize multiple effector proteins to mediate interference, while class 2 systems typically require only a single Cas protein to carry out this process. Types II, V, and VI CRISPR systems recognize a PAM sequence, a short specific sequence near the target site. The PAM sequence helps prevent the CRISPR system from mistakenly targeting the host's DNA [41].

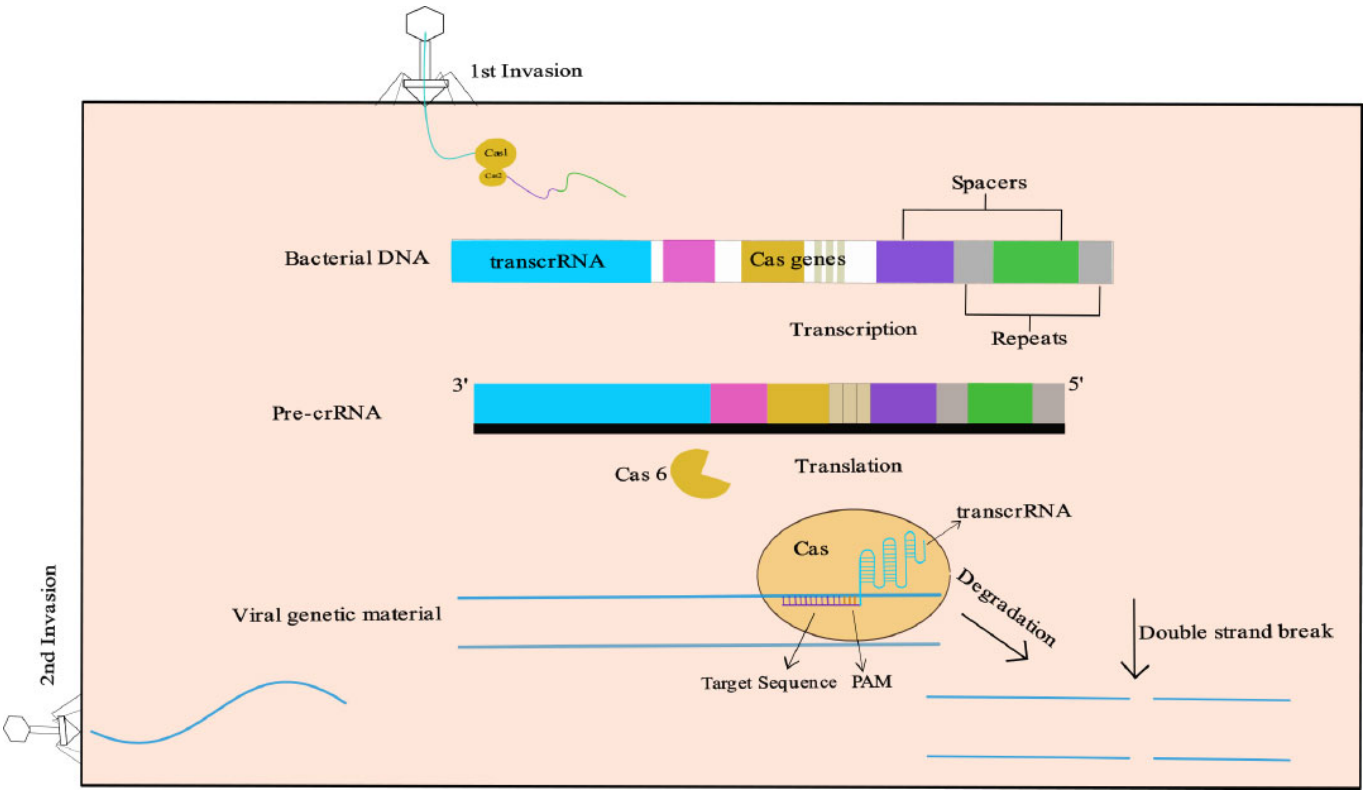

Fig. (1). General mechanism of the CRISPR-Cas system [37].

Table 1. Different CRISPR-Cas systems.

Characteristics	Type I	Type II	Type III	Type IV	Type V	Type VI
Class	Class 1	Class 2	Class 1	Class 1	Class 2	Class 2
Cas protein with nuclease activity	Cas3 and Cas7	Cas9	Cas10	Csf2(Cas5), Csf3(Cas7)	Cas12	Cas13
Target nucleic acid	DNA	DNA	DNA-RNA	Unknown	DNA	RNA
Cleaving pattern	ssDNA	dsDNA breaks blunt ends	Binds RNA to cleave	Unknown	dsDNA breaks staggered ends	RNA
Subtypes	A-E, F1, F2, F3 and G	A to C	A to F	A to E	A - I, K, U and CRISPR-CasΦ	A to B
References	(82–85)	(86–94)	(82–85)	(82,83,95–97)	(86,87,90,91,94,98,99)	(86,87,90,91,94,100,101)

Once the target is located, the interference stage involves the degradation or cleavage of the foreign genetic material. Cas3 protein, for example, plays a role in the cleavage of the targeted sequence. Additionally, the Cas10 protein has a dual function in target cleavage, binding both the crRNA and the target. Other proteins, such as the Csm and Cmr complexes, aid in the interference process for both DNA and RNA targets [37, 38, 41].

5. CRISPR-Cas-BASED DIAGNOSIS OF TUBERCULOSIS

After the infection has occurred, it is very important to diagnose it before the disease worsens. In TB, due to slow growth, a lower bacterial load [paucibacillary TB], difficulty in sampling of Extrapulmonary TB [EPTB], and poor sensitivity and specificity of diagnostic tests lead to a delay in the diagnosis, which ultimately delays the treatment [42].

5.1. CRISPR-Cas9-based Detection

The most widely used CRISPR-Cas system is Cas9, which belongs to the class 2, type II nuclease group [43]. Tram TTB *et al.* used Cas9 with multiplexed sgRNA that targets 52 genes associated with first-line and second-line drug-resistant genes along with IS6110 and IS1081 for identification of the MTBC strain. The diagnostic method utilized the CRISPR-Cas9 system for targeted enrichment followed by Illumina sequencing. After the target enrichment of the samples, the enriched samples were sequenced, which showed high-efficiency reads with a 104X depth, similar to normal culture whole-genome sequencing, which was enhanced to 2076X when the FLASH-TB technique was used. This technique, named Finding Low Abundance Sequences by Hybridization [FLASH], showed high sensitivity and specificity of 100% to Isoniazid [INH], Rifampicin [RIF], Ethambutol [EMB], Kanamycin [KAN], Amikacin [AMK], Moxifloxacin [MOX], Pyrazinamide [PZA], and Streptomycin [STR—only 90% specificity]. The expected Turnaround Time [TAT] is within 48 hours with a cost of $275. Although the method demonstrated high specificity and sensitivity, some small regions like *ddn*, *fbiB*, and *fbiC* had less than 10X coverage during sequencing [44]. Another CRISPR-Cas9-based detection technique is based on an electrochemical biosensor composed of a gold-modified screen-printed carbon electrode [SPCE/Au]. The electrode surface was immobilized with a ferrocene-labeled DNA probe [DNA probe-Fc] with a biotin group at the 3' end, with the help of streptavidin-biotin interaction, followed by blocking non-specific sites with Bovine Serum Albumin [BSA]. This detection tool focuses on mutations in the *katG* gene, which are responsible for INH resistance. The sensor with the immobilized ssDNA hybridizes with the target DNA to form dsDNA. This dsDNA is then recognized and cleaved by the CRISPR-Cas9 system with a gRNA designed to target codon 315, a common mutation site, with the mutation located near the 3' end of the gRNA target sequence for enhanced sensitivity. Due to the cleavage of the dsDNA, a ferrocene signal is produced as detected by Square Wave Voltammetry [SWV]. The biosensor was successfully able to detect DNA in the range of 10^1 to 10^6 Attomolar [aM]. This tool shows promising results for the diagnosis of low-load and resistant bacteria with a TAT of 6 to 7 hours [45].

Although both methods are highly specific and sensitive, they have high TAT, which is a drawback of these methods.

5.2. CRISPR-Cas12a-based Detection

CRISPR-Cas12a, also known as Cpf1, has proven to be a powerful tool for genetic editing. As a versatile genome editing tool with advantages like the self-processing of pre-crRNA into mature crRNA [46], the ability to target T-rich PAM sequences, and the generation of staggered DNA cuts with 5’ overhangs, shows an immense amount of application, especially in the field of detection [47, 48].

Zhang X and team made a detection kit for *Mtb* using CRISPR-Cas12a and Recombinase-Aided Amplification [RAA], which shows prominent specificity and sensitivity towards the identification of *Mtb*. Isothermal amplification was used along with three enzymes, namely recombinase [UvsX], Single-chain Binding Protein [SSB], and DNA polymerase [Klenow, having 3’-5’ exonuclease activity]

[49]. The process was validated by analogizing with the gold standard technique of BACTEC 960 culture results.

The resultant Limit of Detection [LoD] was found to be 3.13 CFU/mL, or about 30 copies/µL. Compared with TAT, Xpert, loop-mediated isothermal amplification, and simultaneous amplification testing, TB-CRISPR took only 90 minutes. This method has overcome the TAT drawback; additionally, TB-CRISPR shows a specificity of 0.946 and a sensitivity of 0.883. The overall cost of the whole assay is about $6.90; compared to other molecular methods, this assay is less expensive [50].

Xiao J *et al.* introduced another assay based on CRISPR-Cas12a using a combination of Graphene oxide-assisted multiplex recombinase polymerase amplification assay [GO-assisted multiplex RPA assay] that targets IS6110 and IS1081 in *Mtb*. The major parts of this assay are GO-assisted singleplex and multiplex RPA, CRISPR-Cas12a-based trans-cleavage, and Lateral Flow Biosensor [LFB]. The DNA from H37Ra was extracted, and GO-assisted singleplex and multiplex RPA were performed. In GO-assisted singleplex and multiplex RPA reactions, the extracted DNA was amplified using RPA. RPA is a well-known method for amplification with a characteristic of high sensitivity, but has a limitation of non-specific amplicon and primer dimer production. Therefore, in this method, GO was used to overcome this drawback, as mentioned by Munawar MA in 2022. After amplification, a trans-cleavage assay was performed with a complex IS6110-gRNA, IS1081-gRNA, and LbCas12a, making it a Cas12a-gRNA complex along with the fluorescence reporter molecule and RPA product. The fluorescence of the complex was detected. To evaluate this, a Lateral Flow Biosensor [LFB] was constructed using a Nitrocellulose [NC] membrane as a reaction platform. On the NC membrane, 3 different areas [conjugated pads] were coated with capture reagents. The first area was coated with Streptavidin-Gold Nanoparticles [SA-GNPs], which were close to the sample loading area; the second area, or the Control Line [CL], was coated with rabbit Anti-Fluorescein Amid [anti-FAM] antibody; and the third area, or the Test Line [TL], was coated with Biotin-Bovine Serum Albumin [biotin-BSA]. The treated samples were loaded along with the running buffer onto the LFB and allowed to flow through the coated NC, similar to a pregnancy kit, where the positive test shows two red lines on CL and TL. This kit was able to reach the LoD of 0.4 copies/µL. The overall process is completed in 60 minutes. It showed a sensitivity of 74.8% and a specificity of 100%. The kit has been approved by the Ethical Committee of Beijing Children's Hospital, Capital Medical University [51]. As the mortality rate among people living with HIV [PLHIV] is high, it is essential to treat such people at the earliest. For adequate treatment of such patients, hypersensitive diagnosis is the most critical part for patients, including children. Hence, Huang Z *et al.* used the LFB assay for the detection of Cell-Free DNA [cfDNA] among these cohorts. This study strengthens and supports the detection kit for pulmonary and extrapulmonary TB detection [52].

With the advancement and a better understanding of the Cas12a system, Jia N *et al.* introduced a novel detection technique. The latest amplification technique, Multiple Cross Displacement Amplification [MCDA] [53], along with CRISPR-Cas12a, was used to detect *Mtb* DNA. This technique consisted of three parts: 1. MCDA primer designing, 2. Standard MCDA assay, and 3. CRISPR-Cas12a-based detection. In MCDA primer designing, ten primers consisted of 2 replacement primers [F_1, F_2], 2 cross primers [CP_1, CP_2], and 6 amplification primers [C_1, C_2, D_1, D_2, R_1, R_2] that target the *sdaA* gene in *Mtb*. Between CP_1 and gRNA [complementary to the P1 primer], a TT base was added as the PAM, as CRISPR-Cas12a is T-rich. A fluorescent label with FAM and Black Hole Quencher [BHQ_1] was designed along with the primers. With the primers, an MCDA assay was performed with the samples and observed using real-time turbidity. Further, the detection was done using a CRISPR-Cas12a-based biosensing system on real-time PCR. The MTB-MCDA-CRISPR assay has 100% specificity for MTB detection. It can detect as low as 40 fg of MTB DNA. The assay has high sensitivity in clinical settings [54].

Since the TAT of the detection increases due to amplification, Wang and colleagues innovated a new technique by eliminating the PCR or isothermal amplification of the DNA. The detection technique consisted of immobilized CRISPR/Cas12a for DNA recognition and cleavage on Graphene Field-Effect Transistors [GFET] for electrical signal amplification. Graphene was used as the detection material, with the extracted DNA introduced onto the Cas12a-GFET. After binding of the *Mtb* DNA to the immobilized Cas12a-GFET, a signal is detected in 5 minutes with a sensitivity of 97.6% and a specificity of 98.6%. The limit of detection that can be detected by this method is about 2.42×10^{-18} M [55].

5.3. CRISPR-Cas12b-based Detection

Another subtype in class 2 type V is Cas12b, also denoted as C2c1. Cas12b has three parts: a nuclease with a single RuvC, a crRNA, and a transcrRNA. Advanced research in this type has revealed that Cas12b has significant applications in human genome editing [43, 56]. With effective targeting, Cas12b is also being used for *Mtb* detection.

With the surge of high-specificity, high-sensitivity, and cost-effective techniques, TB-Quick was introduced by I Kuan Sam *et al.* Enhancing the previous findings and literature of HOLMES [a one-Hour Low-cost Multipurpose highly Efficient System] [57] and HOLMES.v2 [58], based on CRISPR-Cas12b, Kuan Sam *et al.* introduce a detection assay which is a combination of Loop-mediated Isothermal Amplification [LAMP] and CRISPR-Cas12b from *Alicyclobacillus acidoterrestris* [AacCas12b]. Amplification was performed using the most common gene, IS6110, as primers were made for targeting *Mtb* and using the LAMP kit, which contained three pairs of primers. Different temperatures were optimized to amplify the DNA extracted from types of samples, *i.e.*, 65°C for 60 minutes

for pulmonary samples like sputum/BALF DNA and for non-pulmonary samples like cfDNA, 65°C for 80 minutes, and 85°C for 10 minutes for the inactivation of the LAMP reaction [59]. The difference in amplification time is due to a higher concentration of bacilli as compared to non-pulmonary samples. A complete detection assay was performed after amplifying the 16 LAMP products, sgRNA, and Cas12b along with a fluorescence quenching probe [HEX-N12-BHQ1]. The detection was performed in a real-time PCR system, where the fluorescent signals were detected at 48°C for 10 minutes. The assay successfully detects samples containing DNA as low as 1.3 copies/µL. The overall time required for this detection is approximately 1-2 hours depending upon the sample. The specificity and sensitivity of the assay are approximately 95.2% and 86.8%, respectively [59]. In the search for a quick and feasible diagnosis of TB, Peng L and colleagues introduced an assay for fast and early TB diagnosis. TB One-Pot detection assay is a Cross-Priming-based Amplification [CPA] carried out using strand displacement polymerase [60, 61], along with Cas12b derived from *Alicyclobacillus acidophilus* [AapCas12b]. In this assay, as in the previously mentioned assays, Peng L has also used the highly conserved sequence of *Mtb*,*i.e.*, IS6110 and IS1081, which helps target *Mtb*. The fluorescence was observed on a real-time system, and within 32 minutes, the results were obtained. The assay was also checked for cross-reactivity with 15 different Non-Tuberculous Mycobacteria [NTM] strains, namely *M. simiae, M. parascrofulaceum, M. avium, M. intracellulare, M. paraintracellulare, M. kansasii, M. chelonae, M. abscessus, M. triplex, M. lentiflavum, M. paragordonae, M. gordonae, M. scrofulaceum, M. fortuitum, and M. colombiense,* and six common respiratory pathogen strains: *Aspergillus fumigatus, Cryptococcus neoformans, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus*. Observation showed low fluorescence for these strains; on the other hand, the assay showed high fluorescence values for H37Rv and BCG. The assay specificity is 96.7%, and the sensitivity is 67.2%, whereas the LoD for TB One-Pot was 0.8 copies/µl. The combination of CRISPR-Cas12b and CPA has a limitation related to specificity and sensitivity. On comparison of cost with other available tests for *Mtb* detection, this is cost-effective and requires only $1.4 per test, which becomes affordable for the underprivileged [62].

5.4. CRISPR-Cas13-based Detection

CRISPR-Cas13, also known as the C2c2 system, is a subtype belonging to class 2 type VI, as explained previously. This RNA-guided RNA-targeting protein has tremendous applications in gene suppression in mammalian cells. Cas13 extracted from *Leptotrichia wadei* [LwaCas13a] provides the most efficient Cas for gene targeting in mammalian cell lines [63].

Ren W *et al*. developed a diagnostic method using PCR and CRISPR-Cas13a that targeted IS1081, which is a conserved region in MTBC members. Targeting IS1081 showed high sensitivity by detecting the bacterial load of 1 target sequence copy/µl. The sensitivity of 97.4% and high specificity were compared with the gold standard [64].

One more technique for the detection of *Mtb* was done using CRISPR-Cas13a and RAA. IS6110 was used as a target with 100% specificity and no cross-reactivity with NTM. With a limit of detection of 10 copies/µl, the technique showed a sensitivity of 69%. The TAT was 1.5 hours with no false positive test [65].

By using the Cas13 collateral cleavage property, Sri Gowtham Thakku and colleagues created a new platform by improvising the SHERLOCK [Specific High Sensitivity Enzymatic Reporter UnLOCKing] technique [66] to WATSON [Whole Genome Assay using Tiled Surveillance of Nucleic acids] to detect cfDNA. The WATSON technique differs from the SHERLOCK technique in the amplification step, such that in the WATSON technique, the amplification is multiplex tiled-based and targets multiple targets, whereas in the SHERLOCK technique, the amplification is singleplex and targets only one target in the genome. This technique is highly applicable for samples with less concentration of DNA. For this technique, several primers were sequenced for compatibility using an iterative, heuristic search algorithm. After the primers were designed, all 18 crRNAs were synthesized by *in vitro* transcription. DNA from *Mtb*, NTM, and blood samples containing cfDNA was extracted, and a multiplex PCR amplification was performed. The reaction was performed with fluorescent-labelled Cas13, 18 pooled crRNA, and RPA reaction samples. The assay showed a sensitivity of 91% and a specificity of 89%. A Drop Array and a lateral flow assay were performed to confirm the test [67].

With an increasing need for early detection, specific and sensitive diagnostic assays are significant. Diagnosis of TB becomes challenging due to sample contamination, limited sample quantity, reduced growth of the bacteria, interference of NTMs, high cost, etc. Hence, CRISPR-Cas-based diagnosis strengthens the process in all aspects. A tabular summary of all CRISPR-Cas detection is depicted in Table **2**.

6. CRISPR-Cas-BASED DRUG TARGETS FOR ANTI-TUBERCULOSIS DRUGS

Multidrug resistance is a major concern related to TB. The targets for new drugs were identified using two approaches: i) 'Target-to-drug' and ii) 'Drug-to-target.' To target genes, protein mimics essential for bacterial growth and survival were created, and different drug studies were carried out [68]. Another way to target genes involves using homologous gene recombination, phage transduction, and high-density transposon mutant libraries, or knocking down genes by targeting essential genes required for the transcription process. These identification methods fall under the 'Target-to-drug' approach [69].

Table 2. CRISPR-Cas system-based *Mtb* detection assay.

Cas Type	Targets	Amplification	Detection Readout	Sensitivity	Specificity	TAT	Cost	LoD	Advantages	Limitations
Cas9	52 drug resistance genes, IS6110, IS1081	CRISPR enrichment	Illumina sequencing	100% (most drugs)	100% (90% STR)	48 hrs	$275	104X coverage	Drug resistance profiling	Long TAT, low coverage in some regions
Cas9	katG codon 315 (INH resistance)	None	Square wave voltammetry	Detects 10^{1}-10^{6} aM	N/A	6-7 hrs	N/A	10^{1} aM	Ultra-sensitive for mutations	Single mutation only
Cas12a	IS6110	RAA	Fluorescence	88.3%	94.6%	90 min	$6.90	3.13 CFU/mL	Cost-effective, fast	Moderate sensitivity
Cas12a	IS6110, IS1081	GO-assisted RPA	Lateral flow biosensor	74.8%	100%	60 min	N/A	0.4 copies/μL	Visual readout, simple	Lower sensitivity
Cas12a	*sdaA*	MCDA	Real-time PCR fluorescence	High clinical	100%	N/A	N/A	40 fg DNA	Ultra-sensitive	Complex primers
Cas12a	IS1081	None	Graphene FET electrical	97.6%	98.6%	5 min	N/A	2.42×10^{-18} M	No amplification needed	Requires specialized equipment
Cas12a	cfDNA (PLHIV/children)	RPA	Lateral flow	N/A	N/A	N/A	N/A	N/A	EPTB/cfDNA suitable	Limited performance data
Cas12b	IS6110	LAMP	Real-time fluorescence	86.8%	95.2%	1-2 hrs	N/A	1.3 copies/μL	Simple workflow	Temperature optimization needed
Cas12b	IS6110, IS1081	CPA	Real-time fluorescence	67.2%	96.7%	32 min	$1.40	0.8 copies/μL	Cheapest, fastest	Lower sensitivity
Cas13	IS1081	PCR	Collateral cleavage	97.4%	High	N/A	N/A	1 copy/μL	High sensitivity	Requires PCR
Cas13	IS6110	RAA	Collateral cleavage	69%	100%	1.5 hrs	N/A	10 copies/μL	No cross-reactivity	Lower sensitivity
Cas13	Multiplex tiled cfDNA	Multiplex PCR	Fluorescence/drop array/LFA	91%	89%	N/A	N/A	N/A	Genome-wide cfDNA	Complex multiplexing

In the 'Drug-to-target' approach, assay plates such as the microplate Alamar assay or fluorescent-based screening were used for checking cell proliferation against the drug, a process that was time-consuming [70]. CRISPR is not only valuable for discovering new drug targets but also for studying the synergistic or antagonistic relationships between existing tuberculosis drugs and genes. Gene editing in *Mycobacterium* with old techniques was laborious and costly. With this new CRISPR-Cas9 technique, it has become feasible to edit a single gene or multiple genes in an organism. Gene silencing in *Mycobacterium* has become easier with the help of sgRNAs, which have catalytically dead or deactivated nuclease activity. This particular CRISPR interference [CRISPRi] technique targets the gene of interest using the PAM sequence and blocks the RNA polymerase from transcribing the DNA [71].

Choudhary *et al*. utilized this technique to target the *yidC*, *gyrA*, and *engA* genes in *Mycobacterium smegmatis*. In *Mtb*, they targeted *engA*, *ftsZ*, *clpP2*, *groEL1*, *groES*, *clpC1*, and *yidC* genes to assess the efficacy of CRISPRi in both fast- and slow-growing MTBC members [72]. In a study, CRISPRi was used to screen *whiB7*, a transcriptional regulator, as a potential new drug target for the drug clarithromycin, which was repurposed to target the gene [73].

7. VACCINE PRODUCTION

CRISPR-Cas9 has become a time-efficient tool for vaccine development. Time-consuming and labor-intensive knockouts required for live attenuated vaccines have now been simplified with this technique, making it a one-step process. Moraes L. *et al.* created an auxotrophic recombinant BCG strain that expressed a LTAK63 adjuvant [a detoxified subunit of *E. coli* heat-labile toxin] without using antibiotic resistance markers. Using the CRISPR-Cas9 technique *lysA* gene in *Mycobacterium bovis* BCG was knocked out, creating a lysine auxotrophic strain [BCGΔ*lysA*] that cannot grow unless supplemented with the amino acid lysine. The auxotrophic strain was then transformed with a mycobacterial vector carrying both the functional *lysA* gene [to restore growth] and the *ltak63* gene. The *ltak63* gene played the role of a selection marker, replacing the use of antibiotics. rBCGΔ-LTAK63 emerged as a promising TB vaccine candidate, showing reduced bacterial load in the mouse model [74].

8. CHALLENGES In CRISPR-Cas SYSTEM-BASED DIAGNOSIS AND TREATMENT OF TUBERCULOSIS

Advancements in molecular genetics have enhanced the field of diagnosis and treatment. Despite being an advanced technology for diagnosing and treating tuberculosis, CRISPR-Cas cannot fill a few gaps. The limitations related to the diagnosis lie in sample processing, with the long and extensive procedure and the instruments required to process the sample, which require trained personnel. CRISPR-Cas-based assays need to be performed at a very low temperature to avoid denaturation of the protein [75]. In underdeveloped and developing countries, such a diagnosis becomes problematic if not handled with caution and can lead to misdiagnosis. Another disadvantage of CRISPR-Cas-based treatment is the challenge of administering Cas protein. As the Cas proteins are derived from bacteria, they can lead to toxicity and an immune response against them [76]. CRISPR-Cas-based treatment is a new approach in gene therapy [77]. This gene therapy aids in inserting, deleting, or substituting a gene or nucleotide in the mutated position [78]. To introduce the therapeutic genetic material in a living body, two types of vectors are required: i) Viral vector and ii) non-viral vector. The most widely used vectors in gene therapy are viral vectors, namely adenovirus-associated vectors, lentivirus vectors, and full-sized adenoviral vectors [79]. Although the technique has specificity, it still uses gene therapy and has some drawbacks. As *Mycobacteria* survive intracellularly in macrophages, it becomes difficult to target them. Different vectors can be used to transfer this therapeutic genetic material, but these are still under research. In some cases, using these viral vectors proves to be fatal for life and fails during the clinical phases [80]. Another method is the non-viral vector, which includes physical methods as well as chemicals such as lipofectamine, which is a commercially available liposome that can only be used *in vitro,* as they are responsible for high toxicity due to its cationic nature [81]. Hence, it is difficult to deliver CRISPR-Cas with target sgRNA into the human body as a therapeutic agent. Another difficulty with this technology in therapeutics is off-targeting. Although many online bioinformatics tools are available to check for off-targeting, they cannot fully prevent accidental off-target effects caused by mutation. Moreover, the delicate nature of RNA is one more limitation of this technique. The presence of RNase in the surroundings and the secondary structure formation of RNA make it a limiting factor. Although CRISPR-Cas-based assays and therapy offer sensitivity and specificity, they still require trained personnel, instruments, zero or low toxicity/immunogenicity, and low cost related to the administration of the Cas protein, which still presents a gap.

9. DISCUSSION

In the course of END TB strategies, new technologies are being promoted to overcome the limitations and uplift the progress towards eliminating TB by 2030 in the world. With the rising cases of antibiotic resistance, slow growth, and concerns related to HIV and paediatric patients, an easy and faster point-of-care diagnosis and treatment are in demand. After comprehending the CRISPR-Cas system and its unique types along with their ability to specifically target and exhibit nuclease activity, this molecular technique is approachable for detection as well as treatment of TB.

By reducing dependence on sophisticated instruments, the combination of PCR-free amplification along with the CRISPR-Cas system has played a vital role in enhancing sensitivity. Hence, researchers with this combination were

able to successfully diagnose attomolar DNA samples. Although some challenges limit the 100% reliability of CRISPR-Cas in diagnosis and treatment, ongoing research is continuously unveiling the potential of other types and subtypes of CRISPR-Cas systems for various applications in biotechnology and medicine.

CONCLUSION

In summary, in this review, CRISPR-based identification has simplified and accelerated the identification of *Mtb* lineages, enabling researchers to gain a better understanding of the genetic diversity and evolutionary history of this pathogen. These methods hold promise for improving TB diagnosis, treatment, and epidemiological studies, contributing to more effective TB control and management strategies.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to 'Designing of research', 'Collection of data', and 'Paper writing'; these were done by Arti Sharma. Proofreading and analysis were done by Dr. Karuna Gokarn.

LIST OF ABBREVIATIONS

PPD	=	Pure Protein Derivative
WGS	=	Whole Genome Sequencing
TST	=	Tuberculin Skin Test
NAA	=	Nucleic Acid Amplification
SFTP	=	Solitary Fibrous Tumours of the Pleura
SWV	=	Square Wave Voltammetry

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Chai Q, Zhang Y, Liu CH. *Mycobacterium tuberculosis*: An adaptable pathogen associated with multiple human diseases. Front Cell Infect Microbiol 2018; 8: 158. http://dx.doi.org/10.3389/fcimb.2018.00158 PMID: 29868514

[2] Ghebreyesus TA, Kasaeva T, Adam T, *et al*. Global tuberculosis report. 2024. Available from: https://iris.who.int/bitstream/handle/10665/379339/9789240101531-eng.pdf?sequence=1

[3] Koch R. Fortsetzung der Mittheilungen über ein Heilmittel gegen Tuberculose. Dtsch Med Wochenschr 1891; 17(3): 101-2. http://dx.doi.org/10.1055/s-0029-1206198

[4] Nayak S, Acharjya B. Mantoux test and its interpretation. Indian Dermatol Online J 2012; 3(1): 2-6. http://dx.doi.org/10.4103/2229-5178.93479 PMID: 23130251

[5] Mitchison DA. The diagnosis and therapy of tuberculosis during the past 100 years. Am J Respir Crit Care Med 2005; 171(7): 699-706. http://dx.doi.org/10.1164/rccm.200411-1603OE PMID: 15709048

[6] Mirzayev S, Schünemann HJ, Chakaya JM, *et al*. WHO consolidated guidelines on tuberculosis. Module 4: Treatment - Drug-resistant tuberculosis treatmen. 2022. Available from: https://iris.who.int/bitstream/handle/10665/365308/9789240063129-eng.pdf?sequence=1

[7] Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: From the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg 2017; 58(1): E9-E12. PMID: 28515626

[8] Weitzman D, de WEND CAYLEY FE, Wingfield AL. Streptomycin in the treatment of pulmonary tuberculosis. Indian Med J 1951; 45(2): 50-1. PMID: 14823719

[9] Riva MA. From milk to rifampicin and back again: History of failures and successes in the treatment for tuberculosis. J Antibiot 2014; 67(9): 661-5. http://dx.doi.org/10.1038/ja.2014.108 PMID: 25095803

[10] Zein-Eddine R, Refrégier G, Cervantes J, Yokobori NK. The future of CRISPR in *Mycobacterium tuberculosis* infection. J Biomed Sci 2023; 30(1): 34. http://dx.doi.org/10.1186/s12929-023-00932-4 PMID: 37245014

[11] Kostyusheva A, Brezgin S, Babin Y, *et al*. CRISPR-Cas systems for diagnosing infectious diseases. Methods 2022; 203: 431-46. http://dx.doi.org/10.1016/j.ymeth.2021.04.007 PMID: 33839288

[12] Wimmer F, Beisel CL. CRISPR-Cas systems and the paradox of self-targeting spacers. Front Microbiol 2020; 10: 3078. http://dx.doi.org/10.3389/fmicb.2019.03078 PMID: 32038537

[13] Heemskerk D, Caws M, Marais B, Farrar J. Tuberculosis in adults and children. London: Springer 2015. PMID: 26937536

[14] Jensen PA, Lambert LA, Iademarco MF, Ridzon R. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings, 2005. MMWR Recomm Rep 2005; 54(RR-17): 1-141. PMID: 16382216

[15] Balasingham SV, Davidsen T, Szpinda I, Frye SA, Tønjum T. Molecular diagnostics in tuberculosis: Basis and implications for therapy. Mol Diagn Ther 2009; 13(3): 137-51. http://dx.doi.org/10.1007/BF03256322 PMID: 19650669

[16] Desikan P, Panwalkar N, Mirza SB, *et al*. Line probe assay for detection of *Mycobacterium tuberculosis* complex. Indian J Med Res 2017; 145(1): 70-3. http://dx.doi.org/10.4103/ijmr.IJMR_831_14 PMID: 28574017

[17] MacLean E, Kohli M, Weber SF, Suresh A, Schumacher SG, Denkinger CM. Advances in molecular diagnosis of tuberculosis. J Clin Microbiol 2020; 58(10): e01582-19. http://dx.doi.org/10.1128/JCM.01582-19 PMID: 32759357

[18] Aneja A, Nangia V, Chatterji R, Tewari H, Singh A, Bhayana M. Tuberculosis: A common misdiagnosis. Chest 2012; 142(4): 507A. http://dx.doi.org/10.1378/chest.1388932

[19] Nema V. Tuberculosis diagnostics: Challenges and opportunities. Lung India 2012; 29(3): 259-66. http://dx.doi.org/10.4103/0970-2113.99112 PMID: 22919166

[20] Dinnes J, Deeks J, Kunst H, *et al*. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess 2007; 11(3): 1-196. http://dx.doi.org/10.3310/hta11030 PMID: 17266837

[21] Gupta V, Gupta A, Rao NA. Intraocular tuberculosis--An update. Surv Ophthalmol 2007; 52(6): 561-87. http://dx.doi.org/10.1016/j.survophthal.2007.08.015 PMID: 18029267

[22] Bodmer T, Ströhle A. Diagnosing pulmonary tuberculosis with the Xpert MTB/RIF test. J Vis Exp 2012; 3547(62): e3547. http://dx.doi.org/10.3791/3547-v PMID: 22508485

[23] Hopmeier D, Lampejo T, Rycroft J, Tiberi S, Melzer M. The limitations of the Cepheid GeneXpert® Mtb/Rif assay for the diagnosis and management of polyresistant pulmonary tuberculosis. Clin Infect Pract 2020; 7-8: 100038. http://dx.doi.org/10.1016/j.clinpr.2020.100038

[24] Ngabonziza JCS, Decroo T, Migambi P, *et al*. Prevalence and drivers of false-positive rifampicin-resistant Xpert MTB/RIF results: A prospective observational study in Rwanda. Lancet Microbe 2020; 1(2): e74-83. http://dx.doi.org/10.1016/S2666-5247(20)30007-0 PMID: 35544156

[25] Garg A, Agarwal L, Mathur R. Role of GeneXpert or CBNAAT in diagnosing tuberculosis. Med J Dr D Y Patil Vidyapeeth 2022; 15(1): 14-9. http://dx.doi.org/10.4103/mjdrdypu.mjdrdypu_182_20

[26] Nikam C, Jagannath M, Narayanan MM, *et al*. Rapid diagnosis of *Mycobacterium tuberculosis* with Truenat MTB: A near-care approach. PLoS One 2013; 8(1): e51121. http://dx.doi.org/10.1371/journal.pone.0051121 PMID: 23349670

[27] Yadav RN, Singh BK, Sharma SK, *et al*. Comparative evaluation of GenoType MTBDRplus line probe assay with solid culture method in early diagnosis of multidrug resistant tuberculosis (MDR-TB) at a tertiary care centre in India. PLoS One 2013; 8(9): e72036. http://dx.doi.org/10.1371/journal.pone.0072036 PMID: 24039735

[28] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012; 337(6096): 816-21. http://dx.doi.org/10.1126/science.1225829 PMID: 22745249

[29] Makarova KS, Wolf YI, Alkhnbashi OS, *et al*. An updated evolutionary classification of CRISPR–Cas systems. Nat Rev Microbiol 2015; 13(11): 722-36. http://dx.doi.org/10.1038/nrmicro3569 PMID: 26411297

[30] Loureiro A, da Silva GJ. CRISPR-Cas: Converting a bacterial defence mechanism into a state-of-the-art genetic manipulation tool. Antibiotics 2019; 8(1): 18. http://dx.doi.org/10.3390/antibiotics8010018 PMID: 30823430

[31] Ichikawa HT, Cooper JC, Lo L, Potter J, Terns RM, Terns MP. Programmable type III-A CRISPR-Cas DNA targeting modules. PLoS One 2017; 12(4): e0176221. http://dx.doi.org/10.1371/journal.pone.0176221 PMID: 28441427

[32] Goldberg GW, Jiang W, Bikard D, Marraffini LA. Conditional tolerance of temperate phages *via* transcription-dependent CRISPR-Cas targeting. Nature 2014; 514(7524): 633-7. http://dx.doi.org/10.1038/nature13637 PMID: 25174707

[33] Samai P, Pyenson N, Jiang W, Goldberg GW, Hatoum-Aslan A, Marraffini LA. Co-transcriptional DNA and RNA cleavage during type III CRISPR-Cas immunity. Cell 2015; 161(5): 1164-74. http://dx.doi.org/10.1016/j.cell.2015.04.027 PMID: 25959775

[34] Kazlauskiene M, Kostiuk G, Venclovas Č, Tamulaitis G, Siksnys V. A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. Science 2017; 357(6351): 605-9. http://dx.doi.org/10.1126/science.aao0100 PMID: 28663439

[35] Niewoehner O, Garcia-Doval C, Rostøl JT, *et al*. Type III CRISPR–Cas systems produce cyclic oligoadenylate second messengers. Nature 2017; 548(7669): 543-8. http://dx.doi.org/10.1038/nature23467 PMID: 28722012

[36] Rouillon C, Athukoralage JS, Graham S, Grüschow S, White MF. Control of cyclic oligoadenylate synthesis in a type III CRISPR system. eLife 2018; 7: e36734. http://dx.doi.org/10.7554/eLife.36734 PMID: 29963983

[37] Hille F, Charpentier E. CRISPR-Cas: Biology, mechanisms and relevance. Philos Trans R Soc Lond B Biol Sci 2016; 371(1707): 20150496. http://dx.doi.org/10.1098/rstb.2015.0496 PMID: 27672148

[38] Newsom S, Parameshwaran HP, Martin L, Rajan R. The CRISPR-Cas mechanism for adaptive immunity and alternate bacterial functions fuels diverse biotechnologies. Front Cell Infect Microbiol 2021; 10: 619763. http://dx.doi.org/10.3389/fcimb.2020.619763 PMID: 33585286

[39] Silas S, Mohr G, Sidote DJ, *et al*. Direct CRISPR spacer acquisition from RNA by a natural reverse transcriptase–Cas1 fusion protein. Science 2016; 351(6276): aad4234. http://dx.doi.org/10.1126/science.aad4234 PMID: 26917774

[40] Aviram N, Thornal AN, Zeevi D, Marraffini LA. Different modes of spacer acquisition by the *Staphylococcus epidermidis* type III-A CRISPR-Cas system. Nucleic Acids Res 2022; 50(3): 1661-72. http://dx.doi.org/10.1093/nar/gkab1299 PMID: 35048966

[41] Ferretti JJ, Stevens DL, Fischetti VA. Streptococcus pyogenes: Basic biology to clinical manifestations. Oklahoma City: University of Oklahoma Health Sciences Center 2016.

[42] Penz E, Boffa J, Roberts DJ, *et al*. Diagnostic accuracy of the Xpert ® MTB/RIF assay for extra-pulmonary tuberculosis: A meta-analysis. Int J Tuberc Lung Dis 2015; 19(3): 278-284, i-iii. http://dx.doi.org/10.5588/ijtld.14.0262 PMID: 25686134

[43] Shmakov S, Abudayyeh OO, Makarova KS, *et al*. Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. Mol Cell 2015; 60(3): 385-97. http://dx.doi.org/10.1016/j.molcel.2015.10.008 PMID: 26593719

[44] Tram TTB, Ha VTN, Trieu LPT, Ashton PM, Crawford ED, Thu DDA. FLASH-TB: An application of next-generation CRISPR to detect drug resistant tuberculosis from direct sputum. J Clin Microbiol 2023; 61(4): e0163422. http://dx.doi.org/10.1128/jcm.01634-22 PMID: 37010411

[45] Wulandari DA, Zein MIHL, Zakiyyah SN, *et al*. CRISPR-Cas9-based electrochemical biosensor for the detection of *katG* gene mutations in isoniazid-resistant tuberculosis. ADMET DMPK 2025; 13(3): 2766. http://dx.doi.org/10.5599/admet.2766 PMID: 40585408

[46] Fonfara I, Richter H, Bratovič M, Le Rhun A, Charpentier E. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature 2016; 532(7600): 517-21. http://dx.doi.org/10.1038/nature17945 PMID: 27096362

[47] Karvelis T, Gasiunas G, Young J, *et al*. Rapid characterization of CRISPR-Cas9 protospacer adjacent motif sequence elements. Genome Biol 2015; 16(1): 253. http://dx.doi.org/10.1186/s13059-015-0818-7 PMID: 26585795

[48] Zetsche B, Gootenberg JS, Abudayyeh OO, *et al*. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 2015; 163(3): 759-71. http://dx.doi.org/10.1016/j.cell.2015.09.038 PMID: 26422227

[49] Fan X, Li L, Zhao Y, *et al*. Clinical validation of two recombinase-based isothermal amplification assays (RPA/RAA) for the rapid detection of African swine fever virus. Front Microbiol 2020; 11: 1696. http://dx.doi.org/10.3389/fmicb.2020.01696 PMID: 32793160

[50] Zhang X, He X, Zhang Y, *et al*. A new method for the detection of *Mycobacterium tuberculosis* based on the CRISPR/Cas system. BMC Infect Dis 2023; 23(1): 680. http://dx.doi.org/10.1186/s12879-023-08656-4 PMID: 37821806

[51] Xiao J, Li J, Quan S, *et al*. Development and preliminary assessment of a CRISPR–Cas12a-based multiplex detection of *Mycobacterium tuberculosis* complex. Front Bioeng Biotechnol 2023; 11: 1233353. http://dx.doi.org/10.3389/fbioe.2023.1233353 PMID: 37711452

[52] Huang Z, LaCourse SM, Kay AW, *et al*. CRISPR detection of circulating cell-free *Mycobacterium tuberculosis* DNA in adults and children, including children with HIV: A molecular diagnostics study. Lancet Microbe 2022; 3(7): e482-92. http://dx.doi.org/10.1016/S2666-5247(22)00087-8 PMID: 35659882

[53] Wang Y, Wang Y, Ma AJ, *et al*. Rapid and sensitive isothermal detection of nucleic-acid sequence by multiple cross displacement amplification. Sci Rep 2015; 5(1): 11902. http://dx.doi.org/10.1038/srep11902 PMID: 26154567

[54] Jia N, Wang C, Liu X, *et al*. A CRISPR-Cas12a-based platform for ultrasensitive rapid highly specific detection of *Mycobacterium tuberculosis* in clinical application. Front Cell Infect Microbiol 2023; 13: 1192134. http://dx.doi.org/10.3389/fcimb.2023.1192134 PMID: 37287467

[55] Wang W, Du H, Dai C, *et al*. Amplification-free detection of *Mycobacterium tuberculosis* using CRISPR-Cas12a and graphene field-effect transistors. Nanoscale 2025; 17(8): 4603-9. http://dx.doi.org/10.1039/D4NR03852E PMID: 39810563

[56] Shmakov S, Smargon A, Scott D, *et al*. Diversity and evolution of class 2 CRISPR–Cas systems. Nat Rev Microbiol 2017; 15(3): 169-82. http://dx.doi.org/10.1038/nrmicro.2016.184 PMID: 28111461

[57] Li SY, Cheng QX, Wang JM, *et al*. CRISPR-Cas12a-assisted nucleic acid detection. Cell Discov 2018; 4(1): 20. http://dx.doi.org/10.1038/s41421-018-0028-z PMID: 29707234

[58] Li L, Li S, Wu N, *et al*. HOLMESv2: A CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation. ACS Synth Biol 2019; 8(10): 2228-37. http://dx.doi.org/10.1021/acssynbio.9b00209 PMID: 31532637

[59] Sam IK, Chen Y, Ma J, *et al*. TB-QUICK: CRISPR-Cas12b-assisted rapid and sensitive detection of *Mycobacterium tuberculosis*. J Infect 2021; 83(1): 54-60. http://dx.doi.org/10.1016/j.jinf.2021.04.032 PMID: 33951419

[60] Fang R, Li X, Hu L, *et al*. Cross-priming amplification for rapid detection of *Mycobacterium tuberculosis* in sputum specimens. J Clin Microbiol 2009; 47(3): 845-7. http://dx.doi.org/10.1128/JCM.01528-08 PMID: 19116359

[61] Xu G, Hu L, Zhong H, *et al*. Cross priming amplification: Mechanism and optimization for isothermal DNA amplification. Sci Rep 2012; 2(1): 246. http://dx.doi.org/10.1038/srep00246 PMID: 22355758

[62] Peng L, Ma W, Zhong L, *et al*. Diagnostic accuracy of *Mycobacterium tuberculosis* antigen-based skin tests (TBSTs) for tuberculosis infection compared with TST and IGRA: A network meta-analysis. Pathogens 2024; 13(12): 1050. http://dx.doi.org/10.3390/pathogens13121050 PMID: 39770310

[63] Abudayyeh OO, Gootenberg JS, Essletzbichler P, *et al*. RNA targeting with CRISPR–Cas13. Nature 2017; 550(7675): 280-4. http://dx.doi.org/10.1038/nature24049 PMID: 28976959

[64] Ren W, Zhou Y, Li H, *et al*. Development and clinical evaluation of a CRISPR/Cas13a-based diagnostic test to detect *Mycobacterium tuberculosis* in clinical specimens. Front Microbiol 2023; 14: 1117085. http://dx.doi.org/10.3389/fmicb.2023.1117085 PMID: 36819015

[65] Li Q, Wang N, Pang M, *et al*. Rapid and highly sensitive detection of *Mycobacterium tuberculosis* utilizing the recombinase aided amplification-based CRISPR-Cas13a system. Microorganisms 2024; 12(8): 1507. http://dx.doi.org/10.3390/microorganisms12081507 PMID: 39203350

[66] Gootenberg JS, Abudayyeh OO, Lee JW, *et al*. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 2017; 356(6336): 438-42. http://dx.doi.org/10.1126/science.aam9321 PMID: 28408723

[67] Thakku SG, Lirette J, Murugesan K, *et al*. Genome-wide tiled detection of circulating *Mycobacterium tuberculosis* cell-free DNA using Cas13. Nat Commun 2023; 14(1): 1803. http://dx.doi.org/10.1038/s41467-023-37183-8 PMID: 37002219

[68] Singh V, Mizrahi V. Identification and validation of novel drug targets in *Mycobacterium tuberculosis*. Drug Discov Today 2017; 22(3): 503-9. http://dx.doi.org/10.1016/j.drudis.2016.09.010 PMID: 27649943

[69] Alonso MN, Malaga W, Mc Neil M, *et al*. Efficient method for targeted gene disruption by homologous recombination in Mycobacterium avium subspecie paratuberculosis. Res Microbiol 2020; 171(5-6): 203-10. http://dx.doi.org/10.1016/j.resmic.2020.04.001 PMID: 32283218

[70] Abrahams KA, Besra GS. Mycobacterial drug discovery. RSC Med Chem 2020; 11(12): 1354-65. http://dx.doi.org/10.1039/D0MD00261E PMID: 34085044

[71] Rock JM, Hopkins FF, Chavez A, *et al*. Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform. Nat Microbiol 2017; 2(4): 16274. http://dx.doi.org/10.1038/nmicrobiol.2016.274 PMID: 28165460

[72] Choudhary E, Thakur P, Pareek M, Agarwal N. Gene silencing by CRISPR interference in mycobacteria. Nat Commun 2015; 6(1): 6267. http://dx.doi.org/10.1038/ncomms7267 PMID: 25711368

[73] Li S, Poulton NC, Chang JS, *et al*. CRISPRi chemical genetics and comparative genomics identify genes mediating drug potency in *Mycobacterium tuberculosis*. Nat Microbiol 2022; 7(6): 766-79. http://dx.doi.org/10.1038/s41564-022-01130-y PMID: 35637331

[74] Moraes L, Trentini MM, Fousteris D, *et al*. CRISPR/Cas9 approach to generate an auxotrophic BCG strain for unmarked expression of LTAK63 adjuvant: A tuberculosis vaccine candidate. Front Immunol 2022; 13: 867195. http://dx.doi.org/10.3389/fimmu.2022.867195 PMID: 35432328

[75] Ghouneimy A, Mahas A, Marsic T, Aman R, Mahfouz M. CRISPR-based diagnostics: Challenges and potential solutions toward point-of-care applications. ACS Synth Biol 2023; 12(1): 1-16. http://dx.doi.org/10.1021/acssynbio.2c00496 PMID: 36508352

[76] Lou J, Wang B, Li J, *et al*. The CRISPR-Cas system as a tool for diagnosing and treating infectious diseases. Mol Biol Rep 2022; 49(12): 11301-11. http://dx.doi.org/10.1007/s11033-022-07752-z PMID: 35857175

[77] Bunnell BA, Morgan RA. Gene therapy for infectious diseases. Clin Microbiol Rev 1998; 11(1): 42-56. http://dx.doi.org/10.1128/CMR.11.1.42 PMID: 9457428

[78] Uddin F, Rudin CM, Sen T. CRISPR gene therapy: Applications, limitations, and implications for the future. Front Oncol 2020; 10: 1387. http://dx.doi.org/10.3389/fonc.2020.01387 PMID: 32850447

[79] Asmamaw Mengstie M. Viral vectors for the *in vivo* delivery of CRISPR components: Advances and challenges. Front Bioeng Biotechnol 2022; 10: 895713. http://dx.doi.org/10.3389/fbioe.2022.895713 PMID: 35646852

[80] Kumar P, Malik YS, Ganesh B, *et al*. CRISPR-Cas system: An approach with potentials for COVID-19 diagnosis and therapeutics. Front Cell Infect Microbiol 2020; 10: 576875. http://dx.doi.org/10.3389/fcimb.2020.576875 PMID: 33251158

[81] Taha EA, Lee J, Hotta A. Delivery of CRISPR-Cas tools for *in vivo* genome editing therapy: Trends and challenges. J Control Release 2022; 342: 345-61. http://dx.doi.org/10.1016/j.jconrel.2022.01.013 PMID: 35026352

[82] Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol 2017; 37: 67-78. http://dx.doi.org/10.1016/j.mib.2017.05.008 PMID: 28605718

[83] Liu TY, Doudna JA. Chemistry of Class 1 CRISPR-Cas effectors: Binding, editing, and regulation. J Biol Chem 2020; 295(42): 14473-87. http://dx.doi.org/10.1074/jbc.REV120.007034 PMID: 32817336

[84] Xu Z, Chen S, Wu W, Wen Y, Cao H. Type I CRISPR-Cas-mediated microbial gene editing and regulation. AIMS Microbiol 2023; 9(4): 780-800. http://dx.doi.org/10.3934/microbiol.2023040 PMID: 38173969

[85] McBride TM, Cameron SC, Fineran PC, Fagerlund RD. The biology and type I/III hybrid nature of type I-D CRISPR–Cas systems. Biochem J 2023; 480(7): 471-88. http://dx.doi.org/10.1042/BCJ20220073 PMID: 37052300

[86] Tang Y, Fu Y. Class 2 CRISPR/Cas: An expanding biotechnology toolbox for and beyond genome editing. Cell Biosci 2018; 8(1): 59. http://dx.doi.org/10.1186/s13578-018-0255-x PMID: 30459943

[87] Nidhi S, Anand U, Oleksak P, *et al*. Novel CRISPR–Cas systems: An updated review of the current achievements, applications, and future research perspectives. Int J Mol Sci 2021; 22(7): 3327. http://dx.doi.org/10.3390/ijms22073327 PMID: 33805113

[88] Nishimasu H, Ran FA, Hsu PD, *et al*. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell 2014; 156(5): 935-49. http://dx.doi.org/10.1016/j.cell.2014.02.001 PMID: 24529477

[89] Ran FA, Cong L, Yan WX, *et al*. *In vivo* genome editing using Staphylococcus aureus Cas9. Nature 2015; 520(7546): 186-91. http://dx.doi.org/10.1038/nature14299 PMID: 25830891

[90] Mota DS, Marques JM, Guimarães JM, Mariúba LAM. Research Article CRISPR/Cas Class 2 systems and their applications in biotechnological processes. Genet Mol Res 2020; 21(1) http://dx.doi.org/10.4238/gmr18478

[91] Qi LS, Larson MH, Gilbert LA, *et al*. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 2013; 152(5): 1173-83. http://dx.doi.org/10.1016/j.cell.2013.02.022 PMID: 23452860

[92] Mougiakos I, Mohanraju P, Bosma EF, *et al*. Characterizing a thermostable Cas9 for bacterial genome editing and silencing. Nat Commun 2017; 8(1): 1647. http://dx.doi.org/10.1038/s41467-017-01591-4 PMID: 29162801

[93] Hu JH, Miller SM, Geurts MH, *et al*. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 2018; 556(7699): 57-63. http://dx.doi.org/10.1038/nature26155 PMID: 29512652

[94] Mohanraju P, Makarova KS, Zetsche B, Zhang F, Koonin EV, van der Oost J. Diverse evolutionary roots and mechanistic variations of the CRISPR-Cas systems. Science 2016; 353(6299): aad5147. http://dx.doi.org/10.1126/science.aad5147 PMID: 27493190

[95] Pinilla-Redondo R, Mayo-Muñoz D, Russel J, *et al*. Type IV CRISPR–Cas systems are highly diverse and involved in competition between plasmids. Nucleic Acids Res 2020; 48(4): 2000-12. http://dx.doi.org/10.1093/nar/gkz1197 PMID: 31879772

[96] Taylor HN, Laderman E, Armbrust M, *et al*. Positioning diverse type IV structures and functions within class 1 CRISPR-Cas systems. Front Microbiol 2021; 12: 671522. http://dx.doi.org/10.3389/fmicb.2021.671522 PMID: 34093491

[97] Cui N, Zhang JT, Liu Y, *et al*. Type IV-A CRISPR-Csf complex: Assembly, dsDNA targeting, and CasDinG recruitment. Mol Cell 2023; 83(14): 2493-2508.e5. http://dx.doi.org/10.1016/j.molcel.2023.05.036 PMID: 37343553

[98] Safari F, Zare K, Negahdaripour M, Barekati-Mowahed M, Ghasemi Y. CRISPR Cpf1 proteins: Structure, function and implications for genome editing. Cell Biosci 2019; 9(1): 36. http://dx.doi.org/10.1186/s13578-019-0298-7 PMID: 31086658

[99] Tong B, Dong H, Cui Y, Jiang P, Jin Z, Zhang D. The versatile type V CRISPR effectors and their application prospects. Front Cell Dev Biol 2021; 8: 622103. http://dx.doi.org/10.3389/fcell.2020.622103 PMID: 33614630

[100] Al-Shayeb B, Sachdeva R, Chen LX, *et al*. Clades of huge phages from across Earth's ecosystems. Nature 2020; 578(7795): 425-31. http://dx.doi.org/10.1038/s41586-020-2007-4 PMID: 32051592

[101] Abudayyeh OO, Gootenberg JS, Konermann S, *et al*. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 2016; 353(6299): aaf5573. http://dx.doi.org/10.1126/science.aaf5573 PMID: 27256883

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