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Next-generation antimicrobial peptides: Emerging therapeutic strategies against *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis (TB) remains one of the most prevalent infectious diseases worldwide, with drug resistance making treatment increasingly difficult. Recently, antimicrobial peptides (AMPs) have attracted interest as alternative therapeutic agents due to their ability to kill bacteria and regulate the immune system. This review focuses on the potential of AMPs from animal, non-animal, and synthetic origins in the treatment of *Mycobacterium tuberculosis*. This study aimed to evaluate the therapeutic potential of AMPs from animal, non-animal, and synthetic sources against *M. tuberculosis*. A literature review of 54 peer-reviewed studies published between January 2020 and July 2025 was conducted using PubMed, Scopus, Web of Science, and Google Scholar. Keywords related to AMPs and *M. tuberculosis* were used to identify relevant studies. These articles were selected based on their relevance to AMP characteristics, modes of action, and effectiveness against *M. tuberculosis* in laboratory and animal models. AMPs act through a combination of mechanisms: they disrupt the bacterial cell membrane and influence the host's immune response. Peptides from various sources showed promising activity against TB bacteria, whereas the synthetic versions exhibited improved stability and stronger effects. Despite these advantages, several challenges, such as delivery methods, potential toxicity, and the risk of bacterial resistance, remain hurdles for clinical use. As new anti-TB agents, AMPs show great promise, especially for resistant infections. Further research is essential to address current obstacles and to advance these peptides toward safe and effective use in human medicine, offering hope for better TB treatment options in the future.

Keywords: Antibiotic-resistant strains, Drug resistance, Antimicrobial peptides (AMPs), Respiratory infections, Tuberculosis.

Introduction

Mycobacterium tuberculosis (*Mtb*) causes tuberculosis (TB) (Lin *et al*., 2023). The World Health Organization (WHO) estimates that TB is one of the leading infectious causes of illness and death, responsible for approximately 1.5 million deaths each year, particularly in developing countries (Chakaya *et al.,* 2021; WHO, 2022). Despite the availability of effective antimicrobial regimens and a global effort to control TB, the disease continues to pose a significant threat to global health. The primary reasons for this persistence include delayed diagnosis, lengthy treatment regimens, patient non-compliance, and, most critically, the growing emergence of drug-resistant TB strains (Kadhim *et al*., 2023; Mukuka, 2024).

Standard TB treatment includes a combination of four first-line antibiotics—isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA)—administered for an intensive 2-month phase, followed by a continuation phase with INH and RIF for four additional months (Asif *et al.,* 2024; Saeed *et al*., 2024). While this regimen has a relatively high success rate in drug-susceptible TB cases, improper antibiotic use—such as incomplete treatment courses, misdiagnosis, or non-adherence—contributes significantly to the emergence of MDR-TB and extensively drug-resistant TB (Xi *et al.,* 2022). MDR-TB, characterized by resistance to at least INH and RIF, poses substantial treatment challenges due to the need for second-line antibiotics, which are less effective, more toxic, and often expensive (Onu *et al*., 2025). Group A drugs (e.g., bedaquiline, linezolid, and levofloxacin) are prioritized for their high effectiveness. Group B drugs (e.g., clofazimine and cycloserine) serve as important add-ons, whereas Group C drugs are reserved for use when Groups A and B drugs cannot be used. This grouping is based on the efficacy and treatment role, not the

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chemical structure (Mok *et al*., 2022; Li *et al.,* 2025). The limitations of existing treatment regimens and the rapid evolution of drug-resistant TB underscore the urgent need for new therapeutic strategies that are not only effective but also less prone to resistance (Saktiawati *et al*., 2024). Antimicrobial peptides (AMPs) have garnered significant attention in this context. AMPs are small, naturally occurring molecules that form a crucial part of the innate immune system across species (Erdem Büyükkiraz and Kesmen, 2022). They exhibit potent and broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and parasites and have demonstrated efficacy against *Mtb* in several *in vitro* and *in vivo* studies (Sanjana *et al*., 2023; Gupta *et al*., 2025).

AMPs are emerging as promising candidates for a new class of antimicrobials. AMPs exert their antimicrobial effects through multiple mechanisms. These include disrupting microbial membranes via pore formation or micelle-like structures, interfering with intracellular processes such as nucleic acid and protein synthesis, modulating host immune responses, and inhibiting cell wall synthesis (Iranpour *et al*., 2025). Interestingly, some studies suggest that AMPs at sublethal concentrations may induce a hormetic stress response in *Mtb*, potentially enhancing bacterial resilience under certain conditions (Bahl *et al.*, 2025; Jain and Vyas, 2025). This multifaceted mode of action makes it more difficult for pathogens to develop resistance. However, some bacteria, including *Mtb*, have evolved resistance mechanisms under experimental conditions; however, widespread clinical resistance to AMPs has not yet been observed (Oliveira *et al*., 2021; Li *et al*., 2022a,2022b). To overcome these challenges, researchers have focused on engineering more stable and potent synthetic AMP analogs, utilizing chemical modifications such as D-amino acid incorporation, cyclization, or PEGylation to enhance bioavailability, reduce degradation, and maintain antimicrobial activity.

AMPs offer several advantages in the context of TB. Their rapid bactericidal action and low propensity for resistance development make them attractive candidates for therapeutic development (Privalsky *et al*., 2021). Some studies have reported the ability of AMPs derived from neutrophils, macrophages, and epithelial cells to selectively target *Mtb* without affecting host cells (Preethi and Anbarasu, 2023). Furthermore, ongoing innovations in AMP delivery systems, such as nanoparticle encapsulation, liposomal formulations, and inhalable peptides, are addressing challenges related to poor pharmacokinetics (PK) and systemic clearance (Qin *et al.,* 2023).

With the alarming rise of drug-resistant TB and limited progress in developing new antibiotics, AMPs are gaining attention as potential candidates for treating TB (Kumari *et al*., 2025). Although previous reviews have explored the role of peptides in combating *Mtb* and related infections, this review provides a timely and comprehensive update, incorporating literature up to early 2025. This study offers a detailed analysis of recent advancements in AMP classification, mechanisms of action, and modern delivery systems. In contrast to earlier work that focused mainly on natural peptides or select AMP groups, this review also covers synthetic and engineered analogs, highlights their immunomodulatory effects, and examines emerging therapeutic strategies aimed at drug-resistant strains and nontuberculous mycobacteria.

In addition to reviewing recent findings, this study evaluates data from preclinical studies and discusses the main obstacles to clinical application. Covering research from 2000 to early 2025, it aims to assess the potential of AMPs as alternative or adjunctive treatments for TB by exploring how they work, how effective they are in experimental models, and what challenges remain for their translation into practice. This review seeks to support ongoing efforts to develop effective, innovative therapies that can overcome current limitations in TB management by identifying key gaps and summarizing progress across the field.

Study design and search strategy

A focused literature review was conducted to assess the potential of next-generation AMPs in the treatment of *M. tuberculosis* infections. The process adhered to the PRISMA 2020 guidelines to ensure a systematic and transparent approach.

A total of over 50 articles were reviewed based on their relevance and scientific contribution to the subject. The search employed specific keywords such as "peptide," "AMP," and "*M. tuberculosis."* The articles were retrieved from reputable scientific databases, including PubMed, Scopus, Google Scholar, and Web of Science. The search was limited to studies published between January 2020 and July 2025 and to English-language publications.

Inclusion criteria

Studies were included if they were peer-reviewed articles published within the last 5 years (2020–2025) and reported experimental findings on AMPs with activity against *M. tuberculosis*. Eligible studies included *in vitro*, *in vivo*, or clinical investigations that presented original data. Only articles published in English with full-text access were considered.

Exclusion criteria

Studies were excluded if they lacked sufficient methodological detail, did not clearly report AMP activity against *M. tuberculosis*, or presented incomplete or inconsistent data. Only studies that specifically targeted *M. tuberculosis* were included, while those that focused on other bacterial species were excluded.

Data extraction and analysis

For each selected study, information regarding the type of AMP, origin or modification, target strain(s) of *M. tuberculosis*, antimicrobial activity (e.g., Minimum Inhibitory Concentration (MIC) values), mechanism of action, toxicity profiles, delivery systems, and study type (*in vitro*, *in vivo*, or clinical) was extracted.

Findings were compiled and synthesized to provide an overview of the current landscape and future directions of AMPs as potential therapeutic agents against TB.

A total of 99 records were identified through database (n = 89) and register (n = 10) searches. After removing duplicates, 90 records remained for screening. Of these, 62 were excluded based on title and abstract screening. The full texts of 28 articles were assessed for eligibility, and 14 were excluded because they did not meet the inclusion criteria. The remaining 14 studies (10 *in vitro*, 3 *in vivo*, and 1 clinical) were included in the qualitative synthesis. Figure 1 (PRISMA flow diagram) presents a summary of the study selection process.

Analysis of the selected studies

Analysis of the selected studies identified a broad range of AMPs varying in length from 10 to 100 amino acids. These AMPs exhibit significant activity against *M. tuberculosis* through multiple mechanisms, including bacterial membrane disruption, nucleic acid and protein synthesis inhibition, and cell wall biosynthesis interference. In addition to their direct antimicrobial effects, AMPs are known for their broad biological activity, such as immunomodulatory, anti-inflammatory, and wound-healing properties, which may further contribute to their therapeutic potential (Riaz *et al.,* 2024).

Activity of peptides against *M. tuberculosis*

The slow growth and unique cell wall structure of *M. tuberculosis* make treatment difficult (Lin *et al*., 2023). AMPs offer a promising approach because they can attack various parts of the bacteria, such as the cell wall, membranes, and internal components (Magesh *et al.,* 2022; Erriah *et al*., 2025). With antimicrobial resistance expected to cause millions of deaths by 2050, finding new treatments is urgently needed (Fong, 2023). To better illustrate the diversity and therapeutic potential of AMPs against *M. tuberculosis*, a selection of well-characterized AMPs is summarized

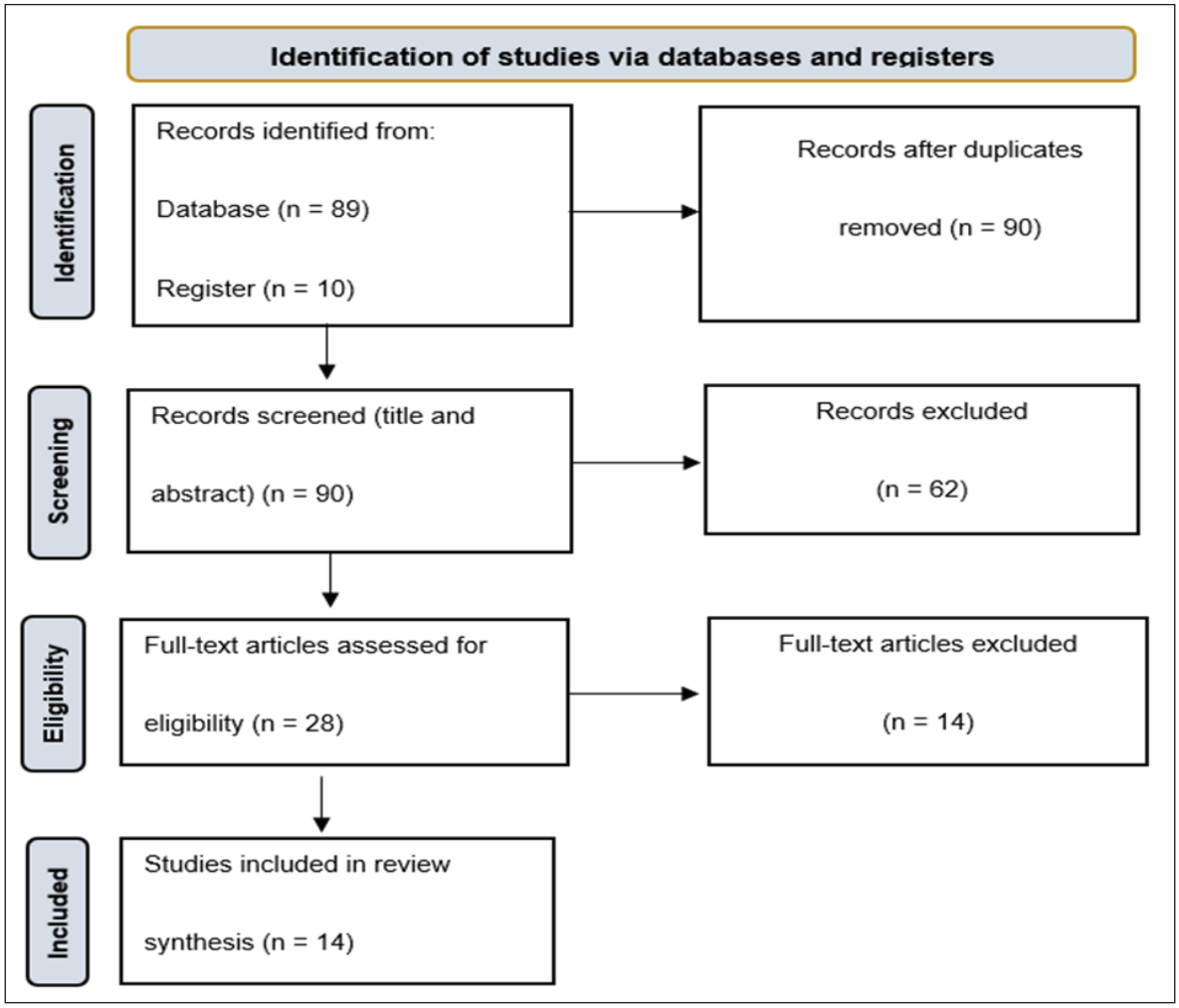

Fig. 1. Flow diagram illustrating the identification, screening, eligibility, and inclusion of studies using PRISMA 2020.

in Table 1 and Figure 2. These peptides were chosen based on their reported efficacy against mycobacterial species, documented mechanisms of action, and relevance in recent experimental or clinical studies. This compilation highlights peptides with varying structural features and targets, offering insights into their potential applications in TB therapy.

A distinctive and disease-causing bacterium

While the majority of mycobacterial species—over 150 identified so far—are found in the environment, only a limited number are capable of infecting both humans and animals. *Mycobacterium tuberculosis* is a strict human pathogen characterized by a low mutation rate and the absence of horizontal gene transfer (Pepperell, 2022). This pathogen co-evolved with humans over thousands of years, and the ability to remain latent for prolonged periods is likely an evolutionary response to the dispersed nature of early human populations. However, there is concern that these latency periods may be shortening (Yang *et al*., 2023).

TB is predominantly a respiratory infection transmitted through airborne particles. Once inhaled, *M. tuberculosis* reaches the lungs and invades macrophages and other immune cells involved in phagocytosis (Kohli, 2022). This encounter initiates a complex immune reaction; however, the bacteria often succeed in establishing long-term persistence within the host. During the initial phase of infection, the host immune system traps the bacteria within granulomas, which are confined lung structures (Muruganandah and Kupz, 2022). At this point, alveolar macrophages—*Mtb*'s favorite cell type—release substances that cause inflammation and send signals to try to get rid of the bacteria (Nisa *et al.,* 2022). Subsequently, the

Table 1. AMPs with reported activity against *M. tuberculosis*, biological sources, antimicrobial profiles, and developmental status.

AMP	Mechanism of action	Source	Stage of development	References
Azurocidin	Disrupts mycobacterial cell wall integrity, inhibits bacterial replication, and promotes phagosome-lysosome fusion.	Human	*In vitro*	Aquino-Domínguez *et al.* (2022)
Lactoferrin	Penetrates mycobacterial membranes via iron transport pathways, disrupting membrane integrity.	Human	Preclinical	Ahmed *et al.* (2021)
Cathepsins	Modulates host immune responses to enhance mycobacterial clearance.	Human	*In vitro*	Anand *et al*. (2022)
Calgranulin	Facilitates phagosome-lysosome fusion to promote bacterial degradation.	Human	*In vitro*	Park *et al.* (2021)
Elastases	Degrades mycobacterial cell wall components and disrupts bacterial membranes.	Human	*In vivo*	Anes *et al*. (2021)
Hepcidin	Contributes to early immune defense by disrupting mycobacterial cell walls and inhibiting bacterial growth	Human	*In vivo*	Park *et al*. (2025)
Defensins	Increases mycobacterial membrane permeability, disrupts cell walls, and suppresses bacterial proliferation.	Human	Preclinical	Fu *et al.* (2023)
Cathelicidin	Inhibits mycobacterial cell wall biosynthesis and promotes structural component breakdown.	Human	Preclinical	Dzurová *et al*. (2024)
Leucine–leucine ratio with 37	Suppresses intracellular Mtb by enhancing macrophage activity via NADPH oxidase 2-dependent mechanisms.	Human	*In vitro and in vivo*	Nisa *et al*. (2022)
Ubiquitinated peptides	Promote mycobacterial cell degradation through intracellular pathways	Host-derived (Human)	*In vitro*	Ma *et al*. (2024)
Antimicrobial RNases	Lysis of mycobacterial cell walls and membranes	Human	*In vitro*	Al-Zubaidi *et al*. (2022)

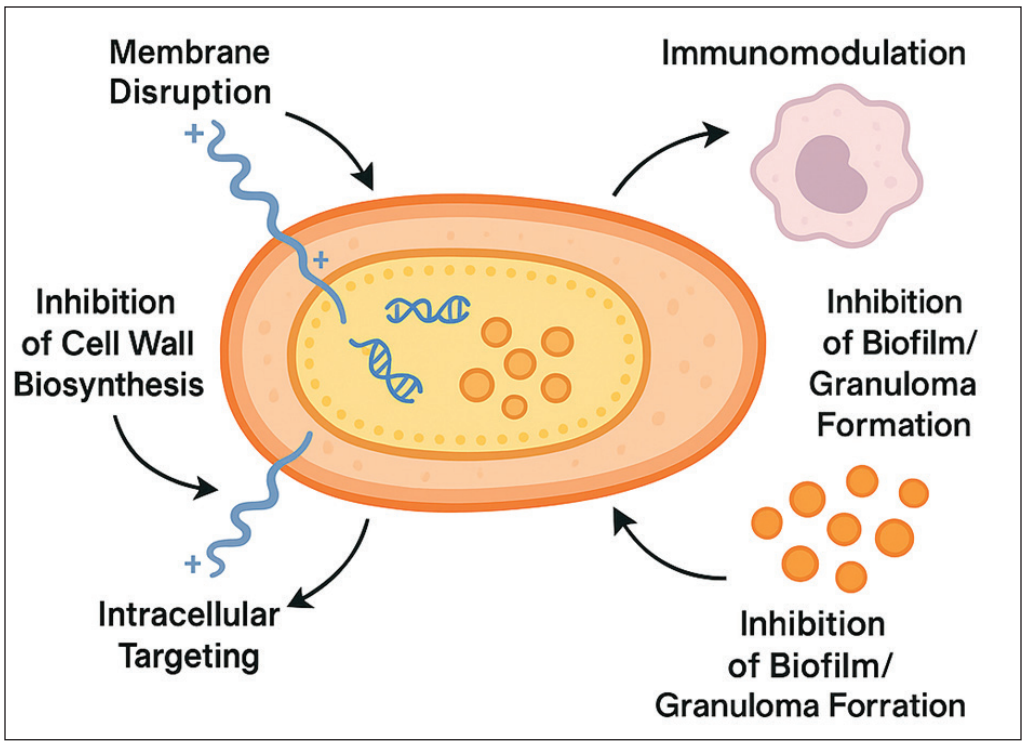

Fig. 2. Major mechanisms through which AMPs exert their effects against *M. tuberculosis.* This figure was designed using Microsoft PowerPoint.

bacilli manipulate the host's gene expression and shift into a dormant state. The granulomas undergo structural changes over time, eventually leading to necrosis (Lyu *et al.,* 2024). This dormant phase helps the bacteria endure within the hostile granulomatous environment and promotes long-term survival inside the host (Sarangi *et al*., 2024). When reactivation occurs, the bacteria resume growth in an extracellular form and begin to form biofilms in lung cavities—biofilms that are particularly rich in drug-resistant cells. The reinfection cycle continues through coughing, which mechanically disrupts granulomas and releases the bacilli back into the air (Paul, 2024).

Exploring the role of AMPs in TB treatment: Mechanisms of action

The increasing resistance to current anti-TB drugs has led to a renewed focus on alternative treatments that were once overlooked, resulting in more research on AMPs. The synthesis of naturally occurring AMPs represents one of the oldest immune defense mechanisms found in living organisms (Erdem Büyükkiraz and Kesmen, 2022). AMPs are effective in small amounts because they work in many ways, come from nature, and are seen as strong options for creating new treatments for tuberculosis (Preethi and Anbarasu, 2023). However, a thorough understanding of the mechanisms behind these innate immune components is essential for effective therapeutic design.

Although AMPs vary widely in amino acid sequence, they often adopt conserved structural motifs, indicating that organisms employ common microbial-killing strategies (Pepperell, 2022). This review highlights the major human AMPs released by innate immune cells in response to mycobacterial infections, focusing on their antimicrobial mechanisms.

In contrast to natural AMPs, synthetic peptides are artificially engineered molecules designed to enhance therapeutic properties, such as stability, specificity, and reduced toxicity (Gan *et al.,* 2021). While natural AMPs often act by disrupting microbial membranes or modulating immune responses, synthetic peptides can be optimized to resist proteolysis, penetrate host tissues more effectively, and selectively target *M. tuberculosis*. Some synthetic AMPs mimic natural sequences, while others are entirely novel, incorporating D-amino acids or non-peptidic backbones to broaden their mechanism of action—such as inhibiting intracellular targets like RNA polymerase or ATP synthase (Brown, 2025). Thus, synthetic peptides offer greater tunability and potential for clinical application, particularly in combating drug-resistant TB, although both natural and synthetic AMPs share antimicrobial potential.

The mycobacterial cell wall

The exceptionally high resistance of mycobacteria to antimicrobial agents is largely attributed to the cell wall's distinctive and intricate structure. This barrier

comprises an elaborate matrix of macromolecules—including peptidoglycan, arabinogalactan, and mycolic acids (collectively forming the MAgP complex)—interwoven with various proteins and polysaccharides, which together form a robust protective layer (Balducci *et al*., 2023).

Mycobacteria cell walls serve as a strong barrier that limits the entry of antimicrobial drugs, making treatment more difficult. In *M. tuberculosis*, a distinctive MAgP complex—covalently bonded—has evolved to support the bacterium's survival inside host cells, enabling it to resist persistent immune attacks and other hostile conditions (Krishnan *et al.,* 2023). Additionally, during the course of infection, the makeup and properties of the cell wall can shift. The structure and chain length of mycolic acids are closely associated with the pathogen's intracellular persistence, positioning them as crucial targets for antibiotic development (Singh *et al.,* 2022). However, the emergence of drug-resistant *Mtb* strains—especially those resistant to INH and EMB, which inhibit mycolic acid synthesis—has driven the search for alternative therapies (Verma, 2024). Resistance has also been observed against PZA, a drug that compromises the integrity of the bacterial cell envelope. Therefore, more focus is being placed on dermcidin—a human peptide released by sweat glands—that can effectively stop mycolyl transferase activity (Hameed, 2024). A key enzyme involved in the biosynthesis of mycolic acids, which are essential components of the *cell wall of M. tuberculosis* (Belete, 2022). Dermcidin disrupts the integrity of the cell wall by targeting this enzyme, impairing the survival of *M. tuberculosis.* Furthermore, there is renewed research focus on targeting peptidoglycan metabolism, another critical component of the bacterial cell wall, as a promising strategy for novel anti-TB therapies (Diab *et al*., 2025).

AMPs compromise the integrity of bacterial cell membranes—either by completely breaking down the lipid bilayer or creating temporary pores (Duong *et al*., 2021). Many AMPs have cationic and amphipathic structures, such as short β-sheets or α-helices, which help them bind to bacterial membranes. The positive charge and hydrophobic nature of AMPs play a key role in determining how they first interact with pathogens (Nur, 2023).

In contrast to eukaryotic cells, which mainly contain negatively charged lipids on the inner side of their membranes, bacterial cells display negatively charged surfaces externally. This distinction allows AMPs to directly kill mycobacteria by disrupting their membranes (Kadhim *et al.,* 2022; Jacobo-Delgado *et al*., 2023).

The cationic AMP residues play a key role in promoting membrane permeabilization. Studies that involve amino acid substitutions in various AMPs have supported this effect. For example, replacing lysine with arginine in variants of Lactoferrin (LF) was found to enhance its mycobactericidal activity (Gagat *et al.,* 2024; Saeed *et al*., 2024) Additionally, while the highly hydrophobic structure of the mycobacterial envelope provides resistance to AMP action, strategies have been developed to increase the proportion of α-helical structure and peptide hydrophobicity, which improve their ability to kill mycobacteria (Preethi and Anbarasu, 2023).

Furthermore, some AMPs directly target surface cell wall proteins, interfering with ion exchange processes and inhibiting mycobacterial growth. These peptides can interact with mycobacterial membrane proteins, such as ATPases, disrupting pH homeostasis within the cell. Interestingly, AMPs that promote membrane permeabilization may also be used as adjuvants to enhance the effectiveness of conventional antibiotics (Chen *et al.,* 2024).

Intracellular targets

While the most well-known AMPs primarily act on the bacterial membrane, an increasing number of peptides have been discovered with previously overlooked alternative targets. Many of these AMPs can cross the membrane, and new research methods are enabling the identification of their interactions with intracellular components (Pirtskhalava *et al*., 2021). AMPs' intracellular targets include nucleic acids, ribosomes, and key metabolic enzymes within *M. tuberculosis*. Some AMPs penetrate macrophages and retain their activity within phagosomes, thereby enhancing intracellular killing. In *M. tuberculosis*, which can survive and replicate within macrophages, the ability of AMPs to reach intracellular compartments is particularly important. Some AMPs can cross host cell membranes, accumulate in phagosomes, and maintain their antimicrobial activity within the hostile intracellular environment (Hossain *et al.,* 2025). For instance, human neutrophil peptides (HNPs) can pass through the lipid bilayer without causing significant membrane disruption and can bind to nucleic acids (Burn *et al*., 2021). Additionally, synthetic AMPs have been developed to selectively target *Mycobacterium* by entering the cells, binding to DNA, and inhibiting processes such as replication and transcription. Remarkably, this intracellular activity can take place even at very low peptide concentrations, reducing the risk of toxicity to host cells (Duarte-Mata and Salinas-Carmona, 2023). Such intracellular targeting is a significant advantage of AMPs over many conventional antibiotics, which often have poor penetration into *M. tuberculosis* macrophage compartments.

Modulation of phagosome-lysosome pathway and autophagy

Mycobacterium tuberculosis has undergone evolutionary adaptations that enable it to persist and replicate within the host macrophages' highly hostile environment. Bacteria can disrupt the normal maturation process of phagosomes, preventing the fusion of phagocytosed material with lysosomes (Allué-Guardia *et al.,* 2021).

Several mechanisms, including the production of reactive oxygen and nitrogen species, acidification of the vacuole, activation of lytic enzymes, and alterations in ion fluxes, are activated in the host cell to eliminate the pathogen (Sharma *et al.,* 2025). *Mycobacterium tuberculosis* not only interferes with the recruitment of vesicular ATPase proton pumps but also blocks the acquisition of markers essential for the endocytic pathway. The bacteria promote fusion with early endosomal vesicles but block fusion with lysosomes, thereby shielding their phagosomal environment from acidification and digestive enzyme action (Rai *et al.,* 2022a, 2022b). Additionally, *M. tuberculosis* inhibits phosphatidylinositol kinase, which decreases phosphatidylinositol triphosphate levels, impairing phagosome maturation. Mycobacterial cell wall structures, such as mannosylated lipoarabinomannan, are thought to be involved in modulating phagosome maturation (Kim *et al*., 2023). These strategies ensure the survival of the pathogen inside the host cell by interfering with the autophagic process at multiple stages (Ge *et al*., 2022).

Several AMPs that promote phagolysosome formation also help eliminate the pathogen. In response, *M. tuberculosis* downregulates AMP expression in macrophages. Autophagy also has protective effects on the host, such as limiting inflammation. Rapamycin is an autophagy activator, and research into novel autophagy-inducing compounds remains a priority (Adikesavalu *et al*., 2021).

Immunomodulatory activities

Immunotherapy plays a crucial role in modern TB treatment strategies. After *M. tuberculosis* is engulfed by alveolar macrophages, its composition is recognized by various mechanisms of detection receptors, triggering signaling pathways that activate immune cells (Zihad *et al*., 2023). In this context, the role of endogenous AMPs is essential for effectively combating infection. AMPs act through two primary mechanisms: direct killing and immune modulation. In direct killing, AMPs target intracellular components, causing membrane rupture or disrupting microbial membranes, ultimately leading to cell lysis. AMPs promote the recruitment and activation of immune cells, such as Interferon -IFN-producing cells, monocytes, and neutrophils, during immune modulation. This enhances pathogen clearance and contributes to controlled inflammation, aiding host defense without excessive immune activation (Kumari *et al*., 2025) (Fig. 3). AMPs can induce both pro-inflammatory and anti-inflammatory responses by releasing a difference in cytokines (Iranpour *et al.*, 2025). Surprisingly, similar AMP may have a pro-inflammatory effect early in the infection and switch to an anti-inflammatory role as the infection progresses (Kumari *et al*., 2025). The production of cytokines, such as interferon gamma (IFNγ), which is crucial for the immune response, is often suppressed by infection (Mehta *et al*., 2022).

Contribution of human endogenous AMPs against TB

Following a mycobacterial infection, human innate immune cells release a range of AMPs into infected tissues. As crucial elements of the non-specific immune response, AMPs have attracted renewed attention as potential therapeutic options. Numerous comprehensive databases—containing curated data on AMP sequences, structures, mechanisms of action, and target organisms—are now available to the scientific community (Cobongela *et al.*, 2022; Preethi and Anbarasu, 2023). These resources support the discovery and development of novel AMPs against resistant pathogens such as *M. tuberculosis*. This section highlights key human AMPs that are reported to be active against TB infection.

Azurocidin

Azurocidin, also known as CAP37, is a 37-kDa cationic antimicrobial protein that is commonly referred to as a heparin-binding protein because of its high affinity for heparin. It acts as an inflammatory mediator by promoting monocyte recruitment to infection sites and increasing vascular permeability. Azurocidin shares structural homology with serine proteases, such as elastase, proteinase 3, and cathepsin G, and exhibits broad antimicrobial activity against various pathogens (Aquino-Domínguez *et al.*, 2022). Notably, cathepsin G contributes to host defense against *M. tuberculosis*, as demonstrated by Anes *et al.* (2021), who reported its role in enhancing antimicrobial responses during pulmonary infection. However, no direct experimental evidence for the activity of azurocidin specifically against *Mycobacterium* species was identified in our literature search. Therefore, its role in antimycobacterial defense remains to be fully elucidated.

Lactoferrin

Lactoferrin is an iron-binding glycoprotein belonging to the transferrin family, composed of 703 amino acids with a molecular weight of approximately 80 kDa. Although it is abundant in milk, neutrophils are the primary source of circulating lactoferrin in the body. It is found in multiple tissues and body fluids and exists in several iron-saturation states, including apo-, monoferric-, and diferric-lactoferrin (Ahmed *et al.,* 2021). Lactoferrin plays a significant role in immune regulation by enhancing interferon-gamma (IFN-γ) expression, promoting the development of IL-17-producing cells in the lungs, and modulating the IL-12/IL-10 cytokine balance to favor a Th1-type immune response (Ghanavi *et al.*, 2021). These immune effects are particularly relevant in the context of *M. tuberculosis* infection. In animal studies, lactoferrin has demonstrated anti-inflammatory effects and increased IL-6 secretion. Larsen *et al*. (2022) reported that Bacillus Calmette–Guérin (tuberculosis vaccine strain) BCG-immunized mice treated with lactoferrin

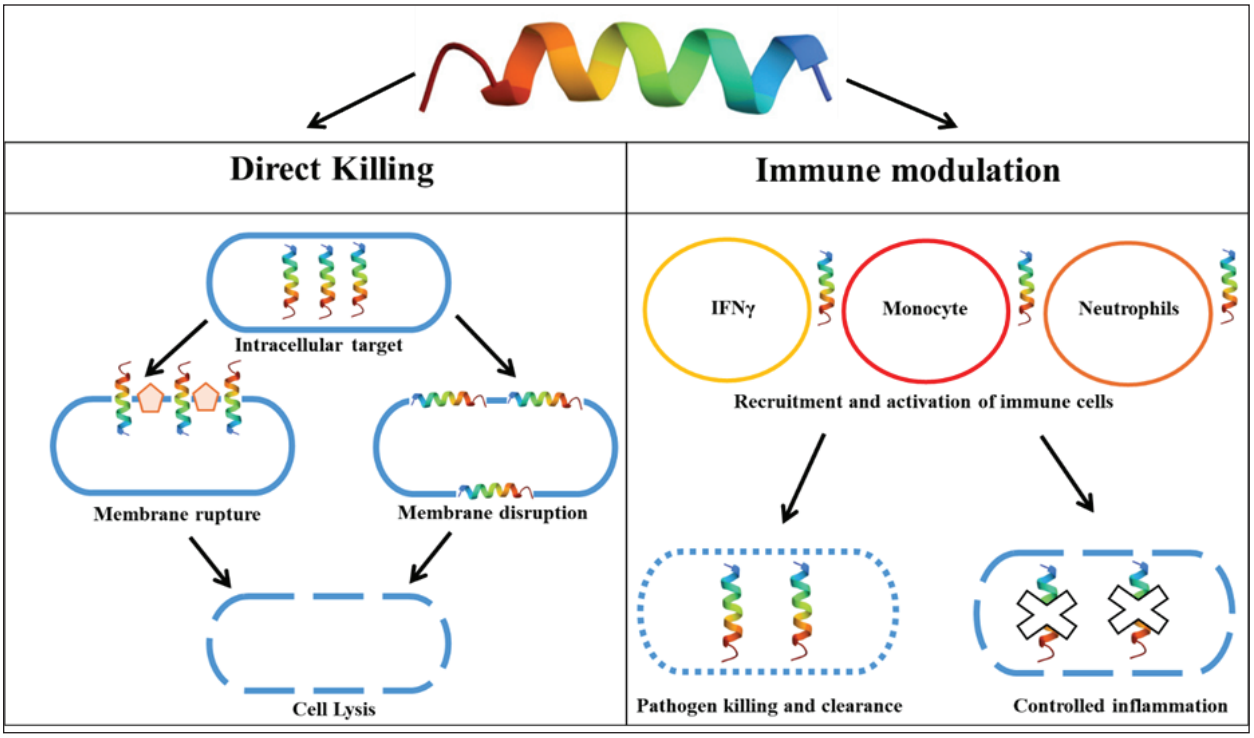

Fig. 3. AMPs in direct killing and immune modulation against *M. tuberculosis*. This figure was designed using Microsoft PowerPoint.

exhibited significantly elevated levels of IFN-γ compared to untreated controls, highlighting BCG's potential as an immunomodulatory agent in TB.

Cathepsins

Cathepsins are grouped into different types based on their enzymatic activity: aspartic proteases include cathepsins D and E; serine proteases include cathepsins A and G; and cysteine proteases encompass cathepsins B, C, F, H, K, L, V, O, S, W, and Z. These peptides are involved in immune functions, such as antigen processing, particularly within lysosomes, where they assist in the elimination of invading pathogens. Cathepsins contribute to host defense mechanisms against *M. tuberculosis*. However, the bacterium enhances its survival inside host cells by suppressing cathepsin expression within macrophages (Anand *et al*., 2022)

Neutrophil elastase (NE)

NE is a serine protease that is released from the azurophilic granules of neutrophils during inflammatory processes. NE plays a crucial role in combating infections, particularly by breaking down outer membrane protein A in gram-negative bacteria. NE also aids in microbial clearance by stimulating macrophage activity and increasing the release of tumor necrosis factor-alpha. According to Tăbăran *et al*. (2020), applying elastase to RAW 264.7 cells improved their intracellular killing efficiency against *Mycobacteria*. Elastases are proteolytic enzymes that can degrade various proteins, including components of bacterial cell walls. In the context of *M. tuberculosis* infection, elastases—particularly those released by immune cells, such as neutrophils—play a defensive role. They contribute to the breakdown of the mycobacterial cell envelope, which is rich in complex lipids and proteins, thereby weakening the immune response of the bacteria. Elastases support the host in controlling and reducing the bacterial load by disrupting the structural integrity of the mycobacteria (Anes *et al*., 2021).

Calgranulin/Calprotectin

Calgranulin, a calcium- and zinc-binding protein, is expressed in granulocytes, macrophages, and squamous epithelial cells. Upon binding to zinc ions (Zn^{2+}), it forms a heterodimer known as calprotectin, which becomes biologically active and exhibits broad-spectrum antimicrobial properties, including activity against *M. tuberculosis* within macrophages (Wang *et al*., 2022a, 2022b). Calprotectin's antimicrobial action is primarily attributed to nutritional immunity, wherein it sequesters essential metal ions such as zinc and manganese, thereby depriving pathogens of the cofactors necessary for enzyme activity, replication, and antioxidant defenses. In particular, *M. tuberculosis* relies on these metals for critical metabolic functions and survival under host-induced stress.

Calgranulin plays a key immunomodulatory role in addition to its direct antimicrobial effect. Dhiman *et al*. (2020) demonstrated that IL-22-mediated inhibition

of mycobacterial growth is associated with increased expression of calgranulin and other calcium-binding proteins, which promote phagolysosomal fusion in infected macrophages (Arshad *et al*., 2020). The zinc-binding capacity of calprotectin contributes to this process by regulating intracellular zinc availability, a crucial factor for phagosome maturation and efficient microbial killing. Therefore, calgranulin supports host defense against *M. tuberculosis* through both metal ion sequestration and IL-22-driven immune response enhancement (Edwards, 2024).

Human neutrophil peptide

Neutrophils are key components of the innate immune system, particularly in the early defense against *M. tuberculosis*. Among their arsenal of antimicrobial agents are α-defensins, also known as HNPs, which are stored in azurophilic granules (Gaffney *et al*., 2022). Eslami and Khosravi (2024) investigated the activity of HNP-1, HNP-2, and HNP-3 against non-tuberculous mycobacteria, including *M. avium* and *M. intracellular*, demonstrating their *in vitro* dose-dependent inhibitory effects on bacterial growth *in vitro*. Furthermore, their study showed that subcutaneous administration of HNP-1 significantly reduced the pulmonary bacterial load in (an inbred laboratory mouse strain: *Bagg Albino, substrain c*) BALB/c mice infected with *M. tuberculosis* H37Rv (1.5×10^4 CFU). This therapeutic effect was associated with enhanced neutrophilic infiltration and local immune activation at the site of infection.

Building on these findings, Wang *et al*. (2022a, 2022b) evaluated the therapeutic efficacy of HNP-1 in a murine TB model, confirming its ability to reduce the mycobacterial burden in infected tissues. Their results indicated that HNP-1 not only exhibits direct antimicrobial effects but may also modulate host immunity, contributing to improved *M. tuberculosis* infection control. These findings highlight the potential of HNPs as adjunctive therapeutics in the treatment of TB.

Hepcidin

Hepcidin is a broad-spectrum AMP primarily synthesized by hepatocytes. It plays a role in innate immunity, particularly through iron regulation and antimicrobial activity (Park *et al*., 2025). Park *et al*. (2025) showed that murine macrophage cultures stimulated with *M. tuberculosis* components and IFN-γ showed a significant increase in hepcidin mRNA expression. However, the present study did not include an *in vivo* infection model or provide direct evidence of increased hepcidin protein levels. The results indicate that hepcidin functions in modulating immunity during mycobacterial infection through an IFN-γ-dependent pathway.

Defensins

Defensins are cysteine-rich cationic peptides categorized into alpha, beta, and theta types. These peptides are produced through the breakdown of prepropeptide and propeptide, forming a mature peptide that exhibits antimicrobial properties and acts on both membrane and intracellular surfaces. HNPs or human neutrophil defensins are divided into α and β types and are derived from neutrophils. Peptides such as HNP-1, beta-defensin-1, and beta-defensin-2 have been shown to effectively kill *M. tuberculosis* both *in vitro* and *in vivo*. These defensins can bind to *M. tuberculosis* cells within the macrophage phagosome, and their role in combating TB infections has been demonstrated in mouse models (Fu *et al.,* 2023).

Cathelicidin

Cathelicidin is an 18-kDa cationic peptide expressed in various cells, such as macrophages, leukocytes, monocytes, myeloid bone marrow cells, and epithelial cells, in response to pathogens (Dzurová *et al*., 2024). The human form, known as human cationic antimicrobial peptide-18, and the murine counterpart, cathelicidin-related AMP, possess antibacterial, chemotactic, and endotoxin-neutralizing properties. These host defense peptides have a conserved N-terminal cathelin domain and a variable C-terminal domain that are crucial for targeting and eliminating a broad range of pathogens, including *M. tuberculosis* (Li *et al*., 2023). Notably, the active form of vitamin D (1,25-dihydroxyvitamin D3) induces cathelicidin expression by activating Toll-like receptors on human macrophages, which triggers the vitamin D receptor and vitamin D-1-hydroxylase enzyme (Abo-Zaid *et al*., 2023). This leads to the induction of autophagy and enhanced intracellular killing of *M. tuberculosis*. Cathelicidin and its active peptide LL-37, derived primarily from human immune cells, exhibit direct antimicrobial activity by disrupting mycobacterial cell membranes and modulating the immune response to strengthen host defenses. While LL-37 targets *M. smegmatis*, *M. bovis* BCG, and *M. tuberculosis* H37Rv by reducing intracellular survival and enhancing innate immunity, several *Bacillus* species have also been reported to produce AMPs with similar antimycobacterial effects, highlighting their potential as natural sources of therapeutic peptides (Preethi and Anbarasu, 2023).

Peptides derived from ubiquitin

Ubiquitin-derived peptides, generated through proteasomal degradation and ubiquitination, function as AMPs. Specifically, peptides conjugated with ubiquitin as a result of proteasomal degradation accumulate in the lysosome, where they can inhibit the growth of *M. tuberculosis* within the autophagolysosome. Although ubiquitin alone is harmless, ubiquitinated peptides, such as Ub2, can penetrate the mycobacterial membrane, potentially disrupting membrane integrity and facilitating intracellular antimicrobial responses—although the exact mechanism remains under investigation (Ma *et al*., 2024).

Human antimicrobial RNases

Human antimicrobial RNases are small secretory proteins (15 kDa) that belong to the RNase A superfamily.

These highly cationic and multifunctional proteins play important roles in host defense, particularly at the mucosal surface. The family comprises eight members expressed in various epithelial tissues and immune cells.

RNase3, also known as eosinophil cationic protein (ECP), is primarily stored in eosinophil granules and, to a lesser extent, in neutrophils. During infection and inflammation, ECP is released and contributes to immune defense. The signal peptide of ECP (ECPsp) has been shown to aid macrophage migration to infection and inflammation sites through pro-inflammatory molecules (Al-Zubaidi *et al*., 2022). Notably, ECP is secreted alongside α-defensin during *M. bovis* BCG infection. Initially, the presence of eosinophils in the respiratory tract during *Mtb* infection was thought to be merely a response to inflammation, but subsequent research has shown that eosinophils, together with neutrophils, actively participate in clearing the infection. TLR2 signaling activates eosinophils through a specific mycobacterial wall component called lipomannan. Both eosinophils and neutrophils release their granule contents into GMs. Eosinophil peroxidase, another protein in eosinophil granules, also exhibits antimycobacterial activity (Malta *et al.*, 2022).

When infected by bacteria, macrophages express two other RNases: RNase6 and RNase7. RNase7, also known as skin-derived RNase, is secreted by keratinocytes and helps protect the skin barrier from various pathogens. Both RNase7 and RNase3 have been shown to eliminate mycobacteria *in vitro*. Recent research indicates that RNases 3, 6, and 7 can also inhibit mycobacterial growth in a macrophage infection model (Castro *et al*., 2021). Given that RNase6 and RNase7 are induced in macrophages during bacterial infection, they likely play a crucial role in fighting intracellular mycobacteria. Furthermore, the immunomodulatory roles of RNases, such as promoting pro-inflammatory cytokine production and attracting dendritic cells, enhance their overall effectiveness (Anguita *et al*., 2024).

Synthetic AMPs

New synthetic AMPs with strong mycobactericidal properties have been developed to fight TB. Synthetic AMP analogs are often seen as the next generation of antibiotics and have attracted significant interest from companies focused on creating new treatments for drug-resistant TB strains. We summarize some of the key synthetic AMPs that have been successfully designed (Table 2), highlighting both their direct antimicrobial effects and host-directed mechanisms.

A widely used strategy in the development of effective AMPs involves designing stable amphipathic α-helices that are rich in specific antimicrobial amino acids (Seyfi *et al*., 2020). These peptides are often modified to resist breakdown by proteolytic enzymes to enhance their performance in living systems, which improves their stability and efficacy. A notable example is the d-LAK peptide family, composed of 25 d-enantiomer amino acids arranged to form a left-handed α-helix and containing eight lysine residues. These peptides are engineered to boost antimicrobial activity while reducing red blood cell toxicity, enabling strong mycobactericidal effects at safe concentrations. D-LAK peptides can also be formulated as a dry powder for inhalation (Wang *et al*., 2023).

Another synthetic peptide, M(LLKK)2M, has demonstrated the ability to combat multidrug-resistant *M. tuberculosis* strains, especially when used alongside RIF. Furthermore, a short synthetic derivative of cathelicidin, known as HHC-10, has proven effective in inhibiting *M. bovis* BCG growth in both *in vitro* assays and *in vivo* models (Jadhav *et al*., 2022).

Remarkably, peptides originating from the N-terminal region of human antimicrobial RNases have shown similar effectiveness in targeting different *Mycobacterium* species to the full-length protein (Bao *et al*., 2023). The RN (1–45) peptides possess a strongly cationic and amphipathic segment that adopts an extended α-helical structure in cell membrane-resembling environments. Furthermore, the RN3(1-45) and RN6(1-45) peptides contain a sequence that encourages bacterial cell agglutination, which provides a distinct mechanistic advantage, potentially aiding microbial clearance at the infection site (Di Natale *et al*., 2020).

Recently, attention has shifted toward a group of short synthetic peptides known as innate defense regulators (IDRs), which possess immunomodulatory properties. These peptides are effective even at very low concentrations, thereby avoiding host toxicity (Tian *et al*., 2021). Rather than killing bacteria directly, IDRs promote the endogenous production of antimicrobial agents by host cells. These peptides can stimulate chemokine release and regulate inflammatory pathways. For instance, IDR-1018 has been successfully tested in a mouse model infected with multidrug-resistant TB via intratracheal administration (Tian *et al*., 2021). Immunoregulatory peptides like IDRs are expected to play a significant role in the treatment of immunocompromised patients very soon.

Several synthetic AMPs have been evaluated for their activity against *M. tuberculosis*, showing promising results in both *in vitro* and *in vivo* models. For example, IDR-1018 exhibits immunomodulatory and antimicrobial effects, with MIC values ranging from 4 to 8 μg/ml. Other engineered peptides, such as DP7 and BMAP-28 analogs, can reduce the intracellular bacterial burden in infected macrophages (Etayash *et al*., 2020). Synthetic AMPs are increasingly delivered via nanocarrier systems, including liposomes and solid lipid nanoparticles, to overcome pharmacokinetic limitations such as rapid degradation and poor bioavailability (van Gent *et al*., 2021). These platforms enhance peptide stability, protect against proteolytic

Table 2. Summary of AMP targets and mechanisms of action against *M. tuberculosis*, highlighting both direct antimicrobial effects and host-directed pathways.

Target site	AMP examples	Mechanism of action	Therapeutic relevance
Cell membrane	LL-37, HNP-1, and Defensins	Disruption of membrane integrity, pore formation, and lysis	Rapid bacterial killing and reduced resistance risk
Cell wall biosynthesis	Cathelicidins, Defensins	Binding to lipid II precursor inhibits PG synthesis	Weakening of cell structure; effective against dormant *Mtb*
Intracellular nucleic acids	IDR-1018, RNase3	Binding or degradation of bacterial DNA or RNA	Inhibition of replication and transcription
The protein synthesis machinery	Synthetic peptides (DP7)	Ribosomal function disruption or interference with translation processes	Prevents bacterial growth and persistence
ATP-binding proteins/ transporters	BMAP-28 analogs	Inhibition of efflux pumps and energy-dependent transport	Enhances drug retention and reduces survival
Metal-ion homeostasis	Calgranulin, Hepcidin	Sequestration of essential metals (Zn, Mn, and Fe)	Starves *Mtb* of nutrients required for survival
Immune modulation (host-directed)	LL-37, IDR-1018, LF	Recruitment and activation of macrophages and autophagy enhancement	Boosts host immunity and supports intracellular pathogen clearance
Autophagic targeting (host mechanism)	Ubiquitinated peptides	Tags *Mtb* for degradation via autophagy	Novel Host-Directed Therapy Component

enzymes, and improve intracellular delivery. Notably, Leucine–Leucine 37 (LL-37)-loaded NPs have demonstrated enhanced retention and antimycobacterial activity in experimental models, highlighting the promise of nanodelivery approaches (Caselli *et al*., 2024).

AMPs stop antimicrobial resistance in TB

Recently, thousands of AMPs have been discovered from natural sources, primarily recognized as critical components of the body's non-specific defense system (Luo *et al*., 2021). Despite the availability of numerous successful antibiotics globally, the demand for new drugs to combat multidrug-resistant mycobacterial strains is growing. Antimicrobial proteins and peptides have great potential for treating mycobacterial infections because they can directly kill bacteria and improve the immune system. However, despite their promising qualities, AMPs still face significant hurdles in entering the pharmaceutical market (Mazurkiewicz-Pisarek *et al*., 2023). One of the major challenges in peptide manufacturing is the high synthesis cost, although some companies are already developing platforms for large-scale peptide production. For instance, recombinant AMPs can be produced in fungi and plants with high yields at relatively low costs. Nevertheless, economic barriers remain substantial. Traditional chemical synthesis, especially for longer or structurally complex peptides, is labor-intensive and expensive, limiting its widespread clinical use. Manufacturing and formulation costs pose significant challenges, particularly in low-resource settings where TB is prevalent (Chaudhary *et al*., 2023).

Recombinant expression systems—using microbes such as *E. coli* or *Pichia pastoris*, as well as transgenic plants and fungi—offer a promising, cost-effective alternative by enabling scalable peptide manufacturing (Yadav *et al*., 2024; Hong *et al*., 2025; Liu *et al*., 2025). Despite this promise, recombinant production presents challenges, including proteolytic degradation by host enzymes, difficulties achieving correct folding or post-translational modifications, and possible toxicity of AMPs to host cells. Advances in genetic engineering, fermentation technologies, and bioprocess optimization are steadily improving the efficiency and reliability of recombinant AMP production, offering a feasible path toward economically sustainable TB therapies. Another limitation of AMP therapy is its vulnerability to proteolytic degradation, particularly when administered systemically. Additionally, the antimicrobial activity of some peptides may be reduced under physiological saline or serum conditions (Wang *et al*., 2021). To address these challenges, new design strategies focus on creating smaller, more affordable analogs with enhanced bacterial target selectivity and broader therapeutic windows.

Various strategies, such as incorporating non-natural amino acids, using backbone mimetics, attaching fatty acids, and modifying the peptide's N- and C-terminal ends, have been devised to improve the bioavailability and stability of peptides within the body. For example, conjugating LL-37 with palmitic acid improves its membrane binding and antimicrobial activity against intracellular bacteria (Kamysz *et al*., 2020). Direct delivery of peptides into the trachea has also been shown to boost their effectiveness in

pulmonary infections. Encapsulating peptides within biodegradable carriers or liposomes—such as LL-37-loaded liposomes or cathelicidin encapsulated in chitosan nanoparticles—has enhanced intracellular uptake and sustained antimicrobial activity against *M. tuberculosis* in infected macrophages (van Gent *et al*., 2021; Caselli *et al*., 2024).

Macrophages naturally uptake these nanodelivery systems, facilitating absorption. Extensive research is ongoing to better understand nanoparticle uptake and intracellular trafficking (Liu *et al*., 2023). A recent innovation involves embedding an LL-37 analog in an HA-based nanogel. This self-assembling polymer system enhances drug delivery by protecting the peptide within its hydrophobic core, thereby significantly improving peptide stability. The nanogel also increases macrophage uptake by approximately 45% and boosts drug-loading capacity by over 60%, thereby improving the overall therapeutic potential (Alford *et al*., 2020).

Despite their promise, AMPs face challenges such as aggregation and poor solubility, which can limit their effectiveness. However, recent advances in predictive computational tools and peptide engineering strategies have enabled the design of AMPs with improved physicochemical properties. Specifically, stabilizing their secondary structures—such as α-helices—has proven effective in enhancing solubility and protecting peptides from enzymatic degradation, thereby increasing their stability and therapeutic efficacy (Lee and Poh, 2023). Predictive tools and strategies are now available to prevent these issues and enhance physicochemical properties. Stabilizing secondary structures further protects AMPs from enzymatic cleavage (Musin and Asyanova, 2025).

Given their critical role in the immune system, AMP expression can be enhanced through immunostimulant therapies, which may bypass some peptide administration limitations. However, some mycobacterial species, including *M. tuberculosis*, can inhibit endogenous AMP production, making supplementation or gene therapy/hormonal induction necessary to achieve optimal therapeutic effects—especially in immunocompromised patients (Peláez Coyotl *et al.,* 2020). The long-term impact of excessive AMP use remains uncertain. Overexposure may lead to the development of resistance mechanisms by mycobacteria, such as alterations in cell envelopes, regulation of macrophage efflux pumps, protease release, or downregulation of host AMP production (Park *et al.,* 2022). Ongoing research focuses on developing new AMP variants, host-directed therapies (HDTs), and combination approaches to combat resistance. Collaborative global efforts such as TBNET and Fight TB continue to advance these strategies, offering hope for more effective treatments against mycobacterial resistance (Gulumbe *et al*., 2024). Implementing AMP stewardship programs and resistance surveillance systems will be essential to monitor emerging resistance patterns and ensure sustainable clinical use.

Off-target effects and mitigation strategies

Although next-generation AMPs show great promise as therapeutic agents against *Mtb,* their potential off-target effects remain a significant challenge that could hinder clinical translation (Preethi and Anbarasu, 2023). These effects refer to unintended interactions between AMPs and host cells or non-target microbial species, which may result in cytotoxicity, immune dysregulation, or disruption of the host's normal microbiota (Ayariga *et al*., 2023). One of the most concerning off-target consequences is the potential cytotoxicity of AMPs toward human cells, particularly erythrocytes and epithelial tissues, due to their membrane-disrupting mechanisms (Rai *et al*., 2022a, 2022b). In addition, some AMPs can exert non-specific immunomodulatory effects, which may lead to excessive inflammation or immune suppression, thereby worsening disease outcomes. Furthermore, interactions with commensal microbiota may contribute to dysbiosis and increase the risk of secondary infections (Saha *et al*., 2024).

Several strategies have been proposed to overcome these challenges. Structural modifications to AMP sequences, such as the incorporation of D-amino acids, peptide cyclization, or the use of peptidomimetic analogs, have been shown to improve target selectivity and reduce toxicity to mammalian cells (Lombardi *et al*., 2025). Delivering AMPs through targeted systems, such as liposomes or polymer-based nanoparticles, can improve localization to infected tissues and minimize systemic exposure. In addition, controlled-release formulations allow the sustained delivery of peptides at therapeutic concentrations, reducing the likelihood of toxicity due to peak dosing. Advanced computational modeling and high-throughput screening have also facilitated the early identification of peptide candidates with lower off-target risks (Vinukonda *et al.*, 2025). Finally, recent approaches have focused on engineering AMPs with refined immunomodulatory activity to promote a balanced host response without triggering harmful inflammation.

A thorough understanding of how AMPs interact with host cells and *Mtb* is essential for achieving an optimal therapeutic balance between efficacy and safety. By combining bioengineering, drug delivery, and immunology innovations, researchers aim to develop safer and more selective AMPs suitable for clinical use against TB.

Comparison with other emerging therapies

AMPs are often compared with other innovative approaches, such as bacteriophage therapy and CRISPR-based antimicrobials, in the search for alternatives to traditional antibiotics (Yeh *et al*., 2022). AMPs offer a unique advantage due to their broad-spectrum activity, rapid bactericidal mechanisms, and immunomodulatory properties, which can enhance host defense. In contrast, phage therapy is highly specific

to bacterial strains and can self-replicate at the site of infection, but may be limited by bacterial resistance and immune clearance. CRISPR-based antimicrobials provide precise gene-targeting capabilities and can disrupt resistance genes, yet they face significant delivery challenges *in vivo* (Palacios Araya *et al*., 2021). While AMPs may suffer from stability, toxicity, and production costs, ongoing peptide engineering and delivery innovations continue to improve their therapeutic potential. Compared with these alternatives, AMPs hold promise as both stand-alone agents and synergistic components in combination therapies for drug-resistant *M. tuberculosis*.

In contrast, **bacteriophage therapy** relies on the use of viruses that specifically infect and lyse bacterial cells. While phages offer highly selective targeting with minimal off-target effects, this specificity can also be a limitation because a single phage may not be effective against diverse clinical isolates. Moreover, the potential for bacterial resistance to phages and challenges related to the host immune system's clearance of phages from circulation may reduce therapeutic efficacy in some cases (Hibstu *et al*., 2022).

CRISPR-based antimicrobials, a rapidly emerging class of gene-editing technologies, provide a novel mechanism to disable essential bacterial genes or resistance determinants with remarkable precision. This specificity allows for the targeted eradication of resistant strains; however, major hurdles remain regarding safe and effective delivery systems, particularly in pulmonary infections such as TB. Delivery vectors must overcome biological barriers and reach infected macrophages, a challenge not yet fully resolved (Allemailem *et al*., 2024).

Despite their promising features, AMPs have limitations, including susceptibility to proteolytic degradation, cytotoxicity at high doses, and high production costs. However, recent advances in synthetic biology and peptide engineering—such as D-amino acid substitution, cyclization, and nanocarrier-based delivery—have shown potential to enhance AMP stability, bioavailability, and reduce toxicity (Al Musaimi *et al*., 2022). Compared with phage or CRISPR therapies, AMPs offer a more immediate translational potential, with several candidates already in preclinical or early clinical evaluation (Zheng *et al*., 2025). Given their broad-spectrum activity and dual antimicrobial-immunomodulatory roles, AMPs may serve not only as standalone agents but also as synergistic partners in combination regimens. This is particularly relevant in the context of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis*, where therapeutic options are limited (Dheda *et al*., 2024). By complementing existing or emerging therapies, AMPs may contribute to a more robust and multifaceted approach to TB treatment in the near future.

Discussion

AMPs represent a unique and promising therapeutic option against *Mtb*, especially considering the growing resistance to conventional anti-TB drugs (Preethi and Anbarasu, 2023). Unlike many standard antibiotics that target a single bacterial process, AMPs display diverse mechanisms that allow them to attack pathogens at multiple levels. Their ability to disrupt bacterial membranes, interfere with essential intracellular components, and modulate the host immune response makes them particularly valuable in combating *Mtb* multidrug-resistant strains (Park *et al.,* 2021).

Despite their potential, AMPs face significant pharmacokinetic challenges that hinder their clinical application. One major limitation is their poor oral bioavailability, mainly due to digestive enzyme degradation and limited absorption across the intestinal epithelium (Azman *et al*., 2022). Systemically administered peptides are frequently rapidly broken down by proteases and cleared by hepatic or renal pathways, resulting in short plasma half-lives and limited therapeutic efficacy. Various strategies have been employed to improve their stability and bioavailability, including peptide cyclization, D-amino acid incorporation, lipid conjugation, and PEGylation. These modifications enhance resistance to enzymatic degradation while maintaining biological activity (Rezende *et al*., 2021).

Advancements in computational tools have provided valuable support for AMP design and optimization. Techniques such as quantitative structure–activity relationship modeling and machine learning allow the prediction of antimicrobial potency and structural behavior (Suay-Garcia *et al*., 2020). *De novo* design platforms and sequence-based algorithms can be used to generate novel AMP candidates with improved selectivity and reduced cytotoxicity (Jin *et al*., 2025). These tools accelerate the development of optimized peptides for preclinical evaluation.

Improving peptide delivery remains a critical area of research. Nanotechnology-based systems—including liposomes, polymeric nanoparticles, and hydrogels—have been successfully explored to enhance the pharmacological profile of AMPs. These carriers can protect peptides from enzymatic degradation, improve their cellular uptake, and enable their controlled release at infection sites. Such delivery systems are particularly important for targeting intracellular pathogens such as *Mtb,* which require efficient penetration into host macrophages (Ayodele, 2024).

Although many AMPs exhibit minimal toxicity at therapeutic doses, safety concerns remain. Depending on their sequence characteristics, certain peptides can display hemolytic or cytotoxic effects, especially high cationicity and hydrophobicity. Sequence modification and encapsulation strategies have helped mitigate these effects in preclinical models (Frederiksen *et*

al., 2020). Additionally, efforts to engineer selective membrane interactions and limit off-target effects have contributed to more favorable safety profiles (Li *et al*., 2022).

Beyond these formulation and design considerations, the successful clinical translation of AMPs must also confront other important barriers. Immunogenicity represents a major concern because repeated administration of peptide-based drugs can trigger unwanted immune responses, potentially reducing efficacy or causing adverse effects. The rapid metabolism and clearance of peptides in human systems further complicate their use, and their behavior *in vivo* can differ markedly from *in vitro* or animal model data (He *et al*., 2023). Furthermore, the absence of AMP-based drugs specifically approved for TB underscores the need for rigorous clinical trials, standardized evaluation methods, and regulatory guidelines to assess their safety and efficacy in humans (Saini *et al*., 2025).

The large-scale production remains another challenge. Natural extraction methods often yield low quantities of peptides, whereas synthetic production is labor-intensive and expensive. However, recombinant expression systems using bacteria, yeast, or transgenic plants offer more sustainable and cost-effective options (Yadav *et al*., 2024; Hong *et al*., 2025; Liu *et al*., 2025). These platforms are especially relevant for AMP production scaling up in resource-limited settings where the TB burden is highest.

Compared with other emerging anti-TB strategies—such as bacteriophage therapy, HDTs, or repurposed small-molecule drugs—AMPs present distinct advantages. Their ability to kill *Mtb* through multiple mechanisms while also modulating the host immune system positions them at the interface between antimicrobial and immunotherapeutic agents (Park *et al*., 2021; Kwon et al., 2023). While bacteriophages offer highly specific bacterial targeting and HDTs aim to boost host resistance, AMPs uniquely combine both antibacterial and immunological functions (Ali *et al*., 2022). Nonetheless, each approach faces its own hurdles, and AMPs may ultimately be most effective when used in combination with other treatments as part of an integrated therapeutic strategy.

Therefore, AMPs are promising candidates for future TB therapies due to their multifunctional activity and low resistance development potential. Recent innovations in the design, delivery, and production of peptides have brought them closer to clinical application. However, continued efforts are needed to overcome immunogenicity, stability, and regulatory challenges to realize their full potential. Collaborative research across molecular biology, pharmacology, and clinical science will be essential for translating AMP-based therapies into effective treatment options for TB and other resistant infections.

Conclusion

Peptide-based treatments for infectious diseases are experiencing renewed interest. AMPs, which are naturally occurring components of the immune system, directly contribute to mycobacterial elimination and often exhibit immunomodulatory properties. Unlike traditional antibiotics, AMPs act through diverse and non-specific mechanisms, making them attractive candidates for pharmaceutical development. However, the efficacy of the BCG vaccine varies widely, and the emergence of extensively drug-resistant *M. tuberculosis* strains represents a critical global health challenge.

Given the limitations of current TB treatments, AMPs are a promising alternative to combating AMR. Preclinical studies provide encouraging evidence of their potential, although clinical validation remains limited. Ongoing research continues to address significant hurdles, including regulatory barriers, poor bioavailability, peptide stability, and large-scale production and cost challenges.

The development of AMPs holds considerable potential to advance TB therapy by integrating novel research methodologies and interdisciplinary approaches. However, further work is necessary to overcome these challenges.

Limitations of the study and future directions

Bacteria have evolved continuously, gaining unique traits that allow them to resist antibiotics. Given this challenge, it is crucial to develop a new generation of antibiotics. However, the limited number of samples and the lack of extensive studies on specific antibiotics and peptides have hindered the progress toward effective treatments. Additionally, the variability in experimental models—such as differences in bacterial strains, host species, dosing regimens, and *in vitro* versus *in vivo* systems—limits the generalizability of the findings. Translating promising *in vitro* results into clinical success remains challenging due to complex host-pathogen interactions and pharmacokinetic factors not fully captured in preclinical studies. Furthermore, the relatively short timeframe of the included studies and potential publication bias may affect the robustness of the conclusions.

Moreover, AMPs face unique challenges that currently limit their clinical application. These include poor stability in physiological conditions, susceptibility to enzymatic degradation, high manufacturing costs, and challenges in achieving effective delivery to infection sites. Furthermore, although AMPs generally show a low propensity for resistance, the potential for resistance development under selective pressure cannot be overlooked. Future research should prioritize the design of more stable and cost-effective AMP analogs, explore advanced delivery platforms such as nanoparticles and inhalable systems, and conduct rigorous *in vivo* and clinical studies to evaluate safety, efficacy, and resistance profiles. These efforts are essential to advance AMPs

from experimental agents to viable therapeutic options against *Mycobacterium* infections.

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