

# Antimicrobial Peptides Against *Mycobacterium Tuberculosis*

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**Abstract:** Tuberculosis is caused by the *Mycobacterium Tuberculosis* which is one of the leading infectious disease and top 10 cause of death worldwide. Thus, a strong need exists for new drugs with special structures and uncommon moles of action to effectively overcome *Mycobacterium Tuberculosis*. Anti-Microbial Peptides (amps) which is short length, cationic and amphipathic peptides have a remarkable strategy for replacement with antibiotics in the treatment of drug resistant infections. A number of recent studies shown that natural AMPS can disrupt the function of *Mycobacterium* cell wall through different modes of action and there after interact with intra cellular targets, including nucleic acids, enzymes and even organelles. This review presents a wide array of Anti- Microbial activities, alongside the associated properties of the AMPS that could be utilised as potential agent in therapeutic schemes of TB treatment.

**Keywords:** Tuberculosis, Drug- resistant tuberculosis, Anti-Microbial Peptides (amps), Anti mycobacterial agent, Toxicity, multi drug resistance (MDR), Extensively drug resistant (XDR)

## 1. Introduction

Tuberculosis is a chronic granulomatous disease and major health problem in developing countries about 1/3 of the world's population is infected with tuberculosis, the causative agent for the TB is *Mycobacterium Tuberculosis* and was first discovered by Robert Koch at the end of the nineteenth century. As per WHO statistics, about 8.6 million people infected with TB in 2012. In addition, there are an estimated 450000 MDR-TB cases and 170000 fatalities occur as a result.

The Revised National Tuberculosis Control Programme (RNTCP) was launched in 1997 and its treatment guidelines have been further revised in 2010. The lack of an effective vaccine poses great problems in the search for new anti-TB drugs. According to their clinical utility the anti-TB drugs are classified as first line and Second line drug. Generally, first line drugs are used most widely because of their high efficacy as well as low toxicity whereas second line drugs have higher toxicity.

First line drugs are Isoniazid (H), Ethambutol (E), Rifampin (R), Streptomycin (S) and Pyrazinamide and the second line drugs include Ethionamide (Eto), Cycloserine (Cs), Terizidone (Trd), Prothionamide (Pto), Fluoroquinolones, Injectable drugs

like kanamycin, Amikacin, Capreomycin etc., Anti Tuberculosis Drugs generally inhibit the synthesis of Mycolic acid present in the cell wall of *Mycobacterium* and distress the cell wall.

Generally, TB can be cured with multidrug chemotherapy, but the long therapy (at least 6 months) has led to poor patient compliance. After several years of trial, the WHO introduced 6-8 Months Multi Drug "Short course" regimens in 1995 under the Dots programme.

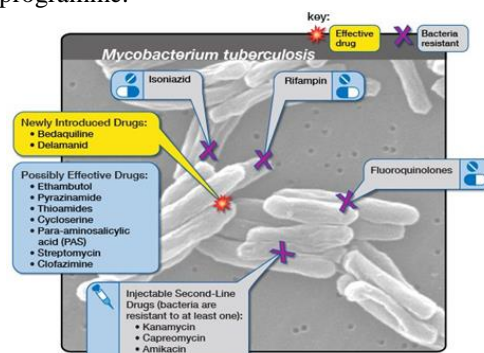


Fig. 1. *Mycobacterium tuberculosis*

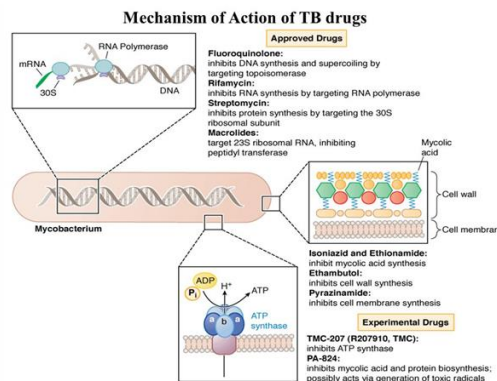


Fig. 2. Mechanism action of tuberculosis drugs

MDR-TB (resistant to at least rifampicin and isoniazid) and XDR-TB (MDR-TB plus resistant to fluoroquinolones and one of the second-line treatments), which occur through spontaneous chromosomal mutation at low frequency, are posing a threat to the control of TB. Therefore, due to the

emergence of drug resistance in M. Tuberculosis against currently available anti mycobacterial therapies, there is an urgent need for the development of new drugs. Several studies have shown that peptides could have many applications in medicine. An important reason for interest in peptides is that they bind to a broad range of biological targets, including in vivo targets, resulting in remarkably high potencies of action and potentially lower toxicity than small molecules. Anti-Microbial Peptides (amps) generally used and are main advantages because of their broad-spectrum antimicrobial activity against Gram-negative and -positive bacteria, fungi, viruses and parasites, and their low probability of induced Resistance in pathogens

Amps are showing the similar mechanism of action as Tuberculosis drugs and are effective against the Mycobacterium which are MDR and XDR and can be used in short course of treatment of TB.

## 2. Structure and general characteristics of AMPS

Antimicrobial peptides are short polypeptides with less than 50 amino acid residues, typically between 15-40 amino acid residues, net positive charge mainly due to an excess of positively charged amino acids arginine, lysine, and histidine, contain 50 % hydrophobic amino acids and constitute an amphipathic structure. In humans, amps activity was initially explored in early 1960's, that cationic peptides were responsible for assisting neutrophils in eradicating bacterial cell. Synthesized in various parts of organisms, they act as regulators and effectors of innate immunity and perform a broad range of activities; such as in the production of chemokine and its release by epithelial and immune cells, exert anti-apoptotic effects on certain immune cells, stimulate wound healing and angiogenesis, and involved in adjuvant activity to increase antibody production. Amps are also able to kill biofilm production, and attract phagocytes chemotactically and induce non-opsonic phagocytosis. Due to their cationic nature, they selectively interact with the negatively charged membrane of microorganism, disturbing the membrane structure. Cationic amps diffused to the lipopolysaccharide and teichoic acid based negatively charged surfaces, in the initial stage as shown in Figure 2. After binding the membrane, they undergo a conformational change, which allows the peptide to translocate into the interior of the bacterial cell.

According to the chemical structures and sequence diversity reported they are categorized into one of the four main structural groups: Linear structure mostly alpha helical peptides,  $\beta$ -strand/sheet peptides having two or more disulfide bridges, extended non-helical linear/sheet peptides rich in Lys, Trp, and His residues, and mixed helical sheet peptides structures (Figure 1). Among these structures, amps adopt mostly alpha helical conformation. Most peptides undergo a transformation from a flexible unstructured structure to a particular structured or fixed conformation when they interact with a membrane. A change in single or double amino acid has

a substantial effect on the secondary structure of peptide that also influences its activity.

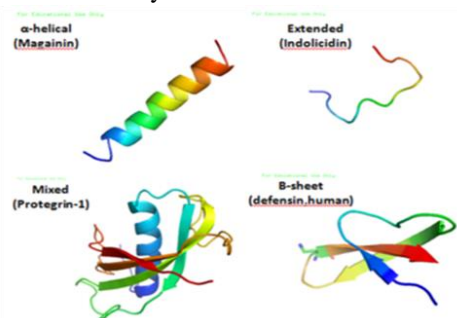


Fig. 3. Structural forms of antimicrobial peptides

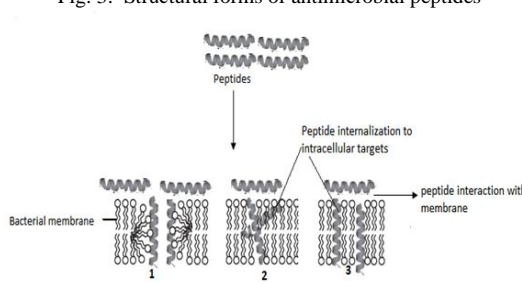


Fig. 4. Antimicrobial peptides mechanism of action

Cationic amps binds the negatively charged bacterial membrane, diffuse and internalize into intracellular targets leading to cell death and use any of these mechanisms.

1. Toroidal pore model
2. Aggregate model of action
3. Barrel-stave.

## 3. Mechanism of action

There are many proposed mechanisms of action for amps, but the exact mechanism is still unclear. A better understanding of the molecular mechanism for the mode of action will assist to develop better drugs. Different studies suggested that their mode of action is predominantly based on their structural features. The hydrophobicity, cationic charge, amino acid sequence, size, influence their interaction with negatively charged bacterial membranes.

A number of studies have reported that amps exhibit strong bactericidal activities through an increased permeability of mycobacterial cell wall. Extensive research has been carried out on the various mechanisms of actions of anti-TB agents; however, their targets and the resistant mechanism are still under study. It is mainly considered that the distinctive cell wall of M. Tuberculosis, which functions as a primary permeability barrier to the entry of anti-TB drugs, contributes to the pathogenicity and resistance. In fact, the unique structure of the mycobacterial cell wall contains a very high concentration of lipid, which makes the cell surface hydrophobic. More specifically, mycolic acids with long hydrocarbon chains characterized as the major constituents of the outer layer play an important role in the lipid-rich cell wall structure. This tightly packed array with extremely low fluidity induces

aggregation of the cells and consequently reduces the permeability of the cell wall.

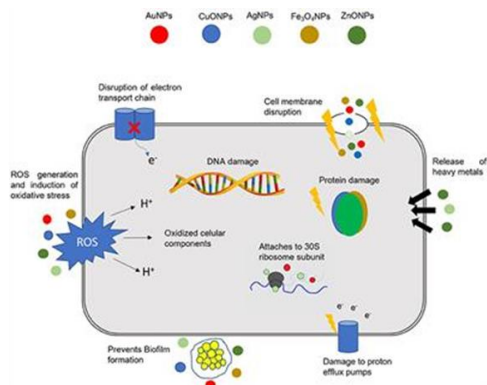


Fig. 5. Mechanism of action

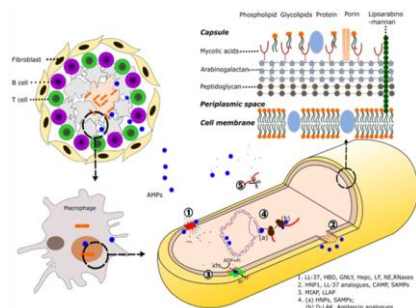


Fig. 6. AMPs

Synthetic AMPS are also using to treat tuberculosis for example adeptantis. To kill mycobacterium as well as other bacteria clinical studies were going on AMPS.

#### 4. Toxicity

It is important to find out if a candidate molecule is toxic for host cells. Toxicity is attributed to high hydrophobicity which leads to hemolysis. Change in hydrophobicity can confer AMP selective activity against microorganism. The ratio of antimicrobial activity and hemolytic activity is expressed as a therapeutic index, so a high therapeutic index is needed to avoid hemolysis of host cells. Therefore, it is crucial to utilize non-hemolytic amps as the seed compounds. Computational methods using knowledge based designs create highly selective amps, based on information from previous known amps, provide a promising strategy to develop high bacterial selectivity peptides. Further advancement in antimicrobial databases would assist researchers to retrieve a vast amount of information on the basis of important peptide parameters such as charge, amino acid content, composition, hydrophobicity to design novel amps.

Gram nature-selective amps can be used to thwart Gram negative bacteria by attacking the outer membrane lipopolysaccharides. These cyclic amps bind and interfere at LPS-binding sites to inhibit membrane synthesis. Fusion peptides are also used to target specific species, providing high

Table 1  
 Important Antimicrobial peptides in clinical trials

Name and peptide sequence	Company	Application	Clinical trial phase, outcome and recent events
Pexiganan (MSI 78)	Genera Plymoth, Dipexium-Pharmaceuticals	Topical Antibiotic	Phase III trials demonstrated no advantage over existing therapies. Another phase III onestep-1 and onestep-2 trials by Dipexium Pharmaceuticals completes for Diabetic foot ulcer in the USA (NCT01594762; NCT01590758)
GIGKFLKK AKKFGKA FVKILKK			
Omiganan	Migenix/ Cutanea-Life Sciences	Severe Acne,	Phase III trials unsuccessful, Phase II showed notable efficacy. Cutanea Life Sciences completes a phase II trial for Acne vulgaris recently (NCT02571998) and plans a phase II trial for Vulvar intraepithelial neoplasia (NCT02596074).Phase II clinical trials as a topical agent in genital wart in Netherland (eudract2015-005553-13)
ILRWPWW PWRRK			
Iseganan ( IB-367)	Ardea Biosciences	Oral mucositis, Pneumoniae	Phase III trials showed no advantage over existing therapies
RGGLCY CRGRFC VCVGR			
Neuprex	Xoma	Impetigo, meningococemia	Phase III trials of meningococemia in children and no development reported yet.
KLFR-(D-naphtho- Ala)-QAK-(D-naphtho- Ala)			
Histatins (P-113)	Demegen	Oral candidiasis	Phase iib trials with candidiasis demonstrated positive results, phase iib trials increase in 34% endpoint efficacy level. Phase III trials not initiated yet.
AKRHHG YKRKFH			
Hif1-11	AM-Pharma	Bacteraemia, Fungal infection	Positive phase I. Under phase II trials for bacterial and fungal infections
GRRRRS VQWCA			
CZEN-002	Zengen	Vulvovaginal Candidiasis	Still no development reported in phase-II clinical trials
(CKPV)2			
OP-145	Leiden University/ Octoplus	Otitis media	Efficacy in phase II trials completed (ISRCTN84220089) but phase III trials still not Initiated
IGKEFKRIVERIK RFLRELVRPLR			
Delmitide	Genzyme	Bacterial infections	Phase II trials completed (ISRCTN84220089).
RXXRX XXGY (X=norleucine)			

selectivity. These fusion peptides comprised of two domains, one provides specific binding to the desired pathogen and the other render bactericidal action. Specificity towards pathogen is based on cell wall structure, membrane receptor, or hydrophobicity. This peptide also selectively eliminates normal flora from pathogenic bacteria. Similarly, protease activated amps and environmental sensing amps have been designed that are activated when virulent proteases are secreted by pathogenic bacteria or sense environment changes like acidic or physiologic pH change respectively. To overcome toxicity and stability issues other techniques are also deployed including; polymeric nanoencapsulation of amps using nanoparticles and nanospheres; pegylating peptides; liposomal formulations and induction of new drug delivery system would strengthen amps therapeutic activity and stability.

### 5. Conclusion

The desperate need to tackle multidrug resistance bacteria has thrived Antimicrobial peptide research to develop them as a new class of antibiotics is highly promising. Their mode of action, broad spectrum activity, ease to synthesize, along with additional biological functions in immune response make them a potential candidate for anti-infective therapeutic development and use. However, there are few limitations that demand attention prior to their clinical use, and only after resolving these issues their significance as an alternative antibiotic would be realized. These factors include high production cost, toxicity, and solubility in physiological conditions. The Scientific community and pharmaceutical companies are working aggressively to cope these issues. Therefore, we are optimistic that with technological advancements, innovative computer-assisted peptide design strategies, high-throughput genomics, and advanced bioinformatic tools will facilitate to identify more cost effective peptide sequences that are highly active without associated toxicity, will significantly boost using amps as next generation therapeutic antibiotics.

The evolution of drug-resistant pathogens has triggered the need to develop novel therapeutic agents. Many studies have provided consistent evidence that antimicrobial host defence peptides display a broad spectrum of activity against bacteria, fungi, plants and viruses. As described in this literature review,

natural peptides with their unique structural architectures are remarkable scaffolds for future drug discoveries. Studying the relationship between peptide structure and function as well as the molecular mechanism of action will lead to a more comprehensive understanding that may be used to design novel compounds with desired activities. Among the amps indicated in Table S1, cyclomarin A, mycobactin S, HNP-1, lariatin A, nocathiacine and DHMP A show the greatest antimycobacterial activity with MIC values  $\leq 4$  mg/L. It is noteworthy that the bacterium has been the most common source of antimycobacterial peptides.

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