Role of Antimicrobial Peptides in Treatment and Prevention of Mycobacterium Tuberculosis: A Review

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Abstract
Tuberculosis (TB) is one of the leading cause of death worldwide, and the world is fighting with this global health emergency from the past 25 year. The current clinical interventions for the management of TB face a number of inherent challenges which includes low patient compliance due to the long therapy regimen, and emerging antimicrobial resistance. Therefore, there is an unmet need of new anti-TB therapeutic agent with enhanced safety profile, which can reduce the duration of therapy, enhanced bioavailability and efficacy against drug resistant forms of TB. Bacteriocins or anti microbial peptides (AMPs) occurring in microbes, human beings and other life forms have been investigated as host defense peptides. Structurally AMPs are short and ionized and play crucial role in innate immunity of host. Some AMPs can kill microbial infections directly while others function indirectly by altering the host defense mechanisms. Amidst rising issue of antibiotic resistance, AMPs are being tested in clinical research as potential antibiotics and novel therapeutics to fight against infections and non-infectious diseases. Studies have also highlighted the ability of AMPs to act against the bacteria spreading tuberculosis. The present review provides information on antimicrobial peptides, highlights their biological role, classification and mode of action in treatment and prevention of tuberculosis. It further mentions the prospects and challenges of developing peptides for their therapeutic applications against mycobacterium tuberculosis.

Keywords Antimicrobial peptides (AMPs) · Tuberculosis · New anti-tuberculosis drugs · Synergistic effect

Abbreviations
TB (Tuberculosis) · AMPS (Antimicrobial peptides) · Mtb (Mycobacterium tuberculosis) · HIV (Human immunodeficiency virus) · WHO (World Health Organization) · Covid-19 (Coronavirus disease) · MDR-TB (Multidrug-resistant TB) · XDR-TB (Extensively drug-resistant TB) · EMB (Ethambutol) · PZA (Pyrazinamide) · DNA (Deoxyribonucleic acid) · CATH-4 (Cathelicidin 4) · DHPS (Di-hydropteroate synthase) · TNF (Alpha tumour necrosis factor alpha) · DOTS (Directly observed treatment Short course) · NETs (Neutrophil extracellular traps) · IP-1 (Iztli peptide 1) · CRAMP (Cathelicidin-related Antimicrobial Peptide) · FDA (Food and Drug Administration) · MIC (Minimal inhibitory concentration) · MMC (Minimal microbicidal concentration)

Introduction
Tuberculosis (TB) is an airborne disorder spread by the bacteria Mycobacterium tuberculosis (Mtb) and mostly affects lungs. TB is one of the leading cause of death worldwide, and for the past 25 years the world has been fighting this global health emergency (Furin et al. 2019). TB may exist as latent (bacteria is asleep or dormant) or active (bacteria is making the person sick) phase depending upon the
immunity of infected person (Lin and Flynn 2010). The presence of conditions such as human immunodeficiency virus (HIV) infection, malnutrition, diabetes, may predispose the individual towards developing active TB (Narasimhan et al. 2013). Co-infection with HIV increases the pathogenicity of Mtb (Bruchfeld et al. 2015). World Health Organization (WHO) data shows that out of 10 million people diagnosed with TB in the year 2019, 1.2 million deaths were reported from HIV-negative individuals and 0.2 million additional deaths were reported from HIV-positive individuals (World Health Organization 2020). The active case load of TB is not uniform throughout the world. High rates of infection are observed in low to middle income countries of Africa and Southeast Asia (World Health Organization 2020). WHO reports suggest that the cumulative reduction in tuberculosis incidence worldwide was 9% from 2015–2019 (from 142 to 130 new cases per 100 000 population), including a reduction of 2.3% between 2018 and 2019. The incidences of TB in India as well as in Southeast Asian region has also seen a declining trend (Fig. 1). TB is mostly curable; however, it can cause death in case the patient does not get proper treatment. Reduced detection of cases results in lack of treatment for infected person, further posing a challenge for the medical community to control death toll. WHO reports that lockdown and restrictions imposed during COVID-19 may cause an additional 1.4 million deaths due to TB between 2020 and 2025 (World Health Organization, 2020).

Research for treatment of TB has been going on from several decades, however till date, Bacille Calmette Guerin (BCG) is the only vaccine available. The vaccine consists of live attenuated strain of bacteria and is successful in controlling infection among children. The vaccine is not effective in adults and the routine treatment options for active TB include use of antibiotics as first line of treatment such as streptomycin, rifampicin, isoniazid, pyrazinamide etc. All these antibiotics have lengthy treatment period, ranging from months to year. Currently, the treatment includes administering cocktail of four different drugs, i.e., isoniazid, pyrazinamide, rifampicin, and ethambutol for 2 months followed by 4-month treatment with rifampicin and isoniazid (Silva et al. 2016a, b). Longer treatment months results in lack of patient compliance and adverse drug interactions which may cause the emergence of drug-resistant TB. Two types of drug resistant TB have been reported i.e., Multidrug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) (Manjelievskaia et al. 2016). MDR-TB cases are treated with second line of treatment options such as fluoroquinolones or injectables (amikacin, capreomycin, and kanamycin) (Rendon et al., 2016). XDR-TB variant shows resistance to first line of drugs as well as to fluoroquinolone and at least one of the injectable of second line treatment (Chan and Iseman 2008) (Table 1). Central Asia and Eastern Europe reports the maximum number of MDR-TB cases (Seung et al. 2015). The 2021 report of WHO indicates that adult men are at highest risk for developing MDR-TB disease, and every year around 3.4% new MDR-TB cases are reported worldwide, while 18% cases are reported in previously treated patients (Shivekar et al. 2020). In 2019, there were 12,350 confirmed cases of XDR-TB (World Health Organization 2020). Drug resistant TB not only increases treatment cost but also causes adverse side-effects. The rise of multidrug-resistant Mtb strains has rendered routine treatment ineffective, underlining the need for novel targeted therapeutic methods (Kurz et al. 2016; Chan and Iseman 2008). AMPs (Antimicrobial peptides) are identified as potential alternative therapy against antimicrobial diseases, such as tuberculosis due to different mode of action (Usmani et al. 2018; Silva et al. 2016a, b; Fox 2013). AMPs participate in innate immunity and act along with cytokines and other host immune modulatory molecules to kill Mtb (Sow et al. 2011). AMPs are 20–60 amino acid biomolecules which play crucial role in innate immunity and can be administered alone or in combination with other drugs for treatment of tuberculosis. The chemical and physical

![Fig. 1 Estimates of TB burden 2020, a India, b South East Asia (Source: WHO)](https://example.com/fig1.jpg)
properties of AMPs allow them to act against bacteria, fungi, and viruses (Table 2) (Arranz-Trullén et al. 2017). This review provides an insight into challenges encountered in treatment of tuberculosis and highlights the need and use of AMPs as therapeutic agents in treating tuberculosis.
Tuberculosis spread by inhaling infectious droplets released as a result of coughing, sneezing, or breathing by an infected active TB patient. The transmission probability depends on the bacterial load in infected sample, the closeness to the person as well as to the duration of exposure (Heemskerk et al. 2015). Once a person is exposed to the bacteria, the immune system of the body reacts to trap bacteria in tubercles or granulomas. Within two years after the primary encounter, the infection progresses to tuberculosis in about 5% of infected patients, while in 10% cases the dormant tubercles get reactivated over the course of several years and the latent infection becomes active TB later in their lifetime (Narasimhan et al. 2013). Studies suggest that one-third of world population suffers from latent TB infection and pose a serious challenge to disease elimination (Dye and Williams 2010). One of the major susceptibility factors of TB include innate and adaptive immune response i.e., alveolar macrophages and dendritic cells. Upon interaction with the pathogen, these immune cells bring about a cascade of signaling pathways activating other immune molecules such as toll-like receptors, heat shock proteins, or inflammatory cytokines (tumor necrosis factors, interleukins, or nitric oxide). However, mycobacterium tuberculosis may evade the macrophage mediated destruction (Sullivan et al. 2012). The local inflammatory milieu stimulates the release of chemokines and cytokines, involved in the mobilization.

### Table 2: Human antimicrobial peptides (AMPs) that aid immune host defense against mycobacteria (Arranz-Trullén et al. 2017)

| AMP                      | Cell type Source                                                                 | Activities                                                                                     | References                                      |
|--------------------------|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------|
| Defensins                | Eosinophils, Macrophages, Epithelial cells, Dendritic cells, Neutrophils          | Formation of membrane pore, Lysis of mycobacterial cell membrane, Inhibition of mycobacterial growth, dendritic and macrophage cells, Regulation of Inflammation and Chemotaxis | (Gallo et al. 2002)                             |
| Cathelicidin             | Epithelial cells, Monocytes, Neutrophils, Dendritic cells, Mast cells, Natural killer cells and Macrophages | Immunomodulation, Pro-inflammatory action, Autophagy activation, Chemotaxis, Neutrophil extracellular traps (NETs), Lysis of mycobacterial cell wall | (Pinheiro Da Silva and MacHado 2012)            |
| Lactoferrin              | Epithelial cells, Neutrophils, Leukocytes                                       | Permeation into bacterial cell Iron channeling, Anti-inflammatory activity                     | (Gallo et al. 2002)                             |
| Azurocidin               | leukocytes and neutrophils                                                        | Lysis of mycobacterial cell wall, Phagolysosomal fusion                                      | (Wiesner and Vilcinskas 2010)                   |
| Hepcidin                 | Macrophages, Hepatocytes and Lymphocytes, Dendritic cells, Lung epithelial cells  | Lysis of mycobacterial cell wall, Mycobacterial regulation and inhibition, Pro-inflammatory activity | (Chaturvedi et al. 2014)                        |
| Granulysin               | Lymphocytes                                                                       | Lysis of mycobacterial cell                                                                  | (Gallo et al. 2002)                             |
| Eosinophil peroxidase    | Eosinophils                                                                        | Lysis of mycobacterial cell wall                                                              | (Wiesner and Vilcinskas 2010)                   |
| Cathepsins               | Neutrophils and Monocytes                                                         | Immunomodulation                                                                              | (Pinheiro Da Silva and MacHado 2012)            |
| Antimicrobial RNases      | Neutrophils and monocytes (RNase6, Eosinophils (RNase3/ECP), Epithelial cells and leucocytes (RNase7) | Lysis of mycobacteria cell wall, Mycobacterial cell agglutination                             | (Pinheiro Da Silva and MacHado 2012)            |
| Elastases                | Macrophages, Neutrophils, Bone marrow cells                                        | Lysis of bacterial cell membrane, Serine protease activity, Chemotaxis and Immunomodulation, Formation of NETs, Macrophage cell agglutination | (Gallo et al. 2002)                             |
| Calgranulin/Calprotectin | Neutrophils, Monocytes, Leukocytes, Keratinocytes                                 | Pro-inflammatory action, Phagolysosomal fusion                                                | (Ross et al. 2001)                              |
| Ubiquitinated peptides   | Macrophages                                                                       | Lysis of mycobacterial cell                                                                   | (Pinheiro Da Silva and MacHado 2012)            |
| Lipocalin2               | Neutrophils                                                                       | Inhibition of mycobacterial growth, Immunoregulation                                           | (Gallo et al. 2002)                             |
of immune cells like lymphocytes, monocytes, neutrophils, and dendritic cells to the infection site, where they form a granuloma with infected macrophages at the center. Studies suggest that elevated levels of tumour necrosis factor alpha (TNF alpha) inhibit MtM development and granuloma formation. The granuloma is a signature pathology of TB, which prevent multiplication of mycobacterium, trapping it inside the granuloma walls, thus leads to the prevention from spreading. The unique feature of this structure is the presence of foam cells formed by the differentiation of chronically activated macrophages (Beham et al. 2011; Szereday et al. 2003) (Fig. 2). Thus, the granuloma forms a necrotic zone at its center that helps the bacteria to emit its spread to different parts of the lungs, exhibiting its transmission via aerosols (Beham et al. 2011). People with HIV infection or those who are immune compromised are at a greater risk of developing active TB.

There are five major challenges to TB treatment; (a) lack of adequate treatment and diagnostic options, (b) lack of patient compliance due to long duration and complicated treatment options, (c) multi-drug resistant TB, (d) HIV co-infection (e) implementation of WHO’s Directly observed treatment short course (DOTS) (Murray, 2006).

**Antimicrobial peptides (AMPs)**

AMPs are cationic, amphipathic in nature, and possess bactericidal activity, which makes them the most effective therapeutic agent against tuberculosis. These anti-microbial peptides have the ability to disrupt normal mycobacterial

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**Fig. 2** Pathogenesis of Tuberculosis (IL-1: Interleukin-1, IL-6: Interleukin-6, IL-12: Interleukin-12, IL-18: Interleukin-18, IFN-γ, Interferon-gamma, TNF-α: Tumour necrosis factor alpha)
cell wall function in a variety of ways, and then interact with intracellular targets as well as nucleic acids, enzymes, and organelles (AlMatar et al. 2018). The probability of antimicrobial resistance with AMPs is low due to several reasons i.e. (a) non-specific mode of killing by AMPs, (b) same molecule possess different killing mechanisms (c) mutations rendering bacteria resistant against AMPs are energy intensive and deleterious in some cases (Silva et al. 2016a, b).

Classification of AMPs

Produced by all life-forms, AMPs are 20–60 amino acid residues and are classified based on their source, activity, structural characteristics, and amino acid-rich species (Huan et al. 2020).

(1) On the basis of source: Antimicrobial peptides can be derived from mammals, amphibians, microorganisms, and insects. Mammal derived AMPs include cathelicidins, defensins, casein, lactoferricin B. Mammalian AMPs are present in human, sheep, cattle, and dairy is one of the most important sources of AMPs (Huan et al. 2020). Among amphibians, frogs are the main source of AMP showing the presence of magainin and skin secretions from frog are also found to be rich source of AMPs (Varga et al. 2018). Another AMP reported from amphibians is cancin. Examples of insect antimicrobial peptides include cecropin and Jellein, while those from microorganisms include nisin and gramicidin (Huan et al. 2020). Another interesting source of AMPs are the marine organisms (Falanga et al. 2016). ASCATH4 (Cathelicidin 4), Myticusin-beta and pardaxin are some of the AMPs obtained from marine environment showing potential immune activity (Huan et al. 2020).

(2) On the basis of activity: AMPs can be classified based on the function i.e., antibacterial (nisin, cecropins, defensins), antiviral (Epi-1), anti-fungal (AurH1), antiparasitic (cathelicidin, temporins, Jellein), antihuman immunodeficiency virus (HIV) (defensins, LL-37, gramicidin D, caerin 1, maximin 3, magainin 2) or anti-tumor peptides (Triptocin, indolicidin) (Huan et al., 2020).

(3) On the basis of structure: Based on structure, AMPs can be classified as linear α-helical peptides, β-sheet peptides, linear extension structure, and both α-helix and β-sheet peptides (Lei et al. 2019).

(4) On the basis of structural characteristics: AMPs can be divided into pole and carpet models based on their structural features. The pole model of AMP can be further divided into toroidal pore and barrel-stave model. In pole models the AMPs are arranged vertical over the cell membrane, whereas in carpet model they lie parallel to the cell membrane. Examples of pole model include magainin-2, lacticin Q, arenicin (Huan et al. 2020).

(5) On the basis of amino-acid rich species: Some antimicrobial peptides are rich in amino acids such as proline (PrAMPs), tryptophan, arginine, histidine, glycine. Examples of amino acid rich AMPs include indolicidin, triptrpcticin, octa-2, HV2, attacins, diptericins (Huan et al. 2020).

AMPs: Mechanism of Action

AMPs play a crucial role in innate immunity and modulate host defense. They possess broad spectrum anti-microbial activity with the ability to kill bacteria, virus, fungi, and parasites (Portell-Buj et al. 2019). Many AMPs have a direct and immediate antibacterial effect by disrupting the integrity of microbial membrane and entering into the cytoplasm of bacteria to react on intracellular targets (Hancock and Diamond 2000). These cationic molecules interact with the anionic surface of microbial membrane, resulting in accumulation of peptide molecules on the surface of membrane (Abedinzadeh et al. 2015). Later the AMPs interact with the cell-wall by forming pore, thinning, altering curvature, localized perturbations or modifying electrostatics (Jenssen et al. 2006; Nguyen et al. 2011; Yeung et al. 2011; Malmsten 2015). This interaction allows the peptide to translocate towards the cytoplasm and interact with intracellular targets i.e., nucleic acids, enzymes, organelles (Giuliani et al. 2007). Due to their amphipathic nature, as indicated in bacterial membrane, AMPs can interact in aqueous and lipid surroundings too (Sohlenkamp and Geiger 2015). AMPs show increased permeability to mycobacterial cell wall (Portell-Buj et al. 2019).

The interaction of AMPs with mammalian cell membrane is quite different from the bacterial membranes. The reason lies in different composition and structure of the mammalian membrane. The mammalian phospholipids are primarily zwitter ionic resulting in net neutral charge, while bacterial membranes have negatively charged outer leaflets (Ebenhoe et al. 2014). In mammalian membranes, phospholipids are distributed asymmetrically, with zwitterioneric phospholipids in the outer leaflet and negatively charged phospholipids from cytoplasm in the inner leaflet (Lai and Gallo 2009; Zasloff 2002). As a result, interactions between AMPs and mammalian cell membranes are mostly mediated by hydrophobic contacts, which are weak in comparison to electrostatic interactions between AMPs and bacterial membranes. In addition, unlike microorganisms, mammalian cell membranes have significant cholesterol content which stabilizes the phospholipid bilayer and decrease the activity of AMPs (Lai and Gallo 2009).
Because of these structural differences between the membranes, the peptides selectively act on bacterial cells rather than human cells, making them a potential therapeutic to be used against microbes (Matsuzaki 2009) (Fig. 3). The following section discusses the role of AMPs in pathogenesis and elimination of tuberculosis.

**Role of AMPs in Elimination of Tuberculosis**

Till now, it has become quite clear to us as how AMPs attacks only bacterial cells. This section highlights the possible role of AMPs in tuberculosis.

AMPs interact with the host cells by penetrating in the cells or by modulating host immune response. Several studies show the efficacy of AMPs in inducing autophagy for bacterial death and clearance, e.g., three antimicrobial peptides including Indolicidin induced autophagy mediated cell death in the protozoa *Leishmania* together with disruption of cell membrane (Bera et al. 2003). The ability of autophagy induction and bacterial clearance makes AMP a potential therapeutic for treatment of tuberculosis.

In order for AMPs to be beneficial, it is crucial that they act at defined concentrations without affecting the host cells. A synthetic AMP, IP-1 (Iztli peptide 1) was reported to induce autophagy by sequestering ATP and helps in elimination of Mtb due to antimicrobial activity (Peláez et al. 2020). Vitamin-D induced AMP cathelicidin (LL-37) acts as anti-infective agent against Mtb (Chung et al. 2020). LL-37 is the active form of cationic AMP cathelicidin and is known to show activity against intra and extracellular Mtb (Deshpande et al. 2020). LL-37 is highly expressed in case of pulmonary tuberculosis and interacts with macrophages and Mtb. Mtb are phagocytosed by macrophages and prevents their degradation by inhibiting formation of acidic phagolysosomes. However, the super resolution microscopy study suggests that this inhibition may be suppressed by LL-37 (Deshpande et al. 2020). The results show that LL-37 is internalized in macrophages with the help of clathrin or lipid-raft. Once inside the infected macrophages, LL-37 enters the phagosomes and brings about degradation of Mtb via cell wall degradation or by inducing cellular degradation. In another study, in-silico analysis was carried out to understand the

![Fig. 3](image)

Fig. 3 Representation of AMP-mediated bacterial killing pathway
inhibitory effect of dermcidin AMP on bacterial cell wall component mycolic acid (Banerjee and Gohil, 2016). Mycolic acid is hydroxy fatty acids found in cell wall of Mycobacteria taxon including Mtb and are known to be crucial for the pathogenicity of bacteria and their existence. Dermcidin on the other hand is an AMP secreted by sweat glands and displays broad spectrum antimicrobial activity against both gram positive and gram-negative pathogenic bacteria. The results of the study show that dermcidin inhibits transfer of mycolic acid to cell wall and interferes with cell wall synthesis (Banerjee and Gohil 2016). Hepcidin is another AMP produced by human liver cells which shows broad spectrum antimicrobial activity (Sow et al. 2011). Studies have shown that hepcidin is localized to mycobacteria-containing phagosomes and shows antimicrobial activity against Mtb. Further, Mtb infection stimulates expression of hepcidin mRNA by innate immune cells in lungs (Sow et al. 2011). Hepcidin expression and induction in immune response highlights the role of AMP in clearing *mycobacterium tuberculosis*.

**Microbial Resistance to AMPs**

Antimicrobial peptides like any other protein structure are susceptible to proteases which may bring about their degradation. Certain features of AMPs prevent the microbes to develop resistance against them such as: (a) the concentration of AMPs at the infection site is strictly regulated by its expression, (b) secondly the production of various AMPs occur simultaneously, (c) thirdly the cost of developing resistance against AMPs is high for microbes, (d) fourthly lack of specific target epitopes in structure of AMPs make it difficult for microbes to degrade AMPs and (e) finally the AMPs belong to different structural classes of peptides and destroying single peptide will not result in resistance (Lai and Gallo 2009). There are other structural variations as well, i.e., the presence of disulphide bridges in AMPs such as defensins, protegrin’s, cryptidin-related sequence, make these peptides resistant to proteolysis (Peschel and Sahl 2006). Apart from the presence of disulphide bridges, other features such as thioether bridges, introduction of proline residues and amidation of protease-susceptible C terminal make antimicrobial peptides more resistant towards proteolysis. Further, considering that AMP’s major target is the bacterial cell membrane, it will be complicated for microorganisms to maintain its cell membrane’s functional and structural integrity, while evading AMPs’ membrane-disrupting effects (Lai and Gallo 2009).

However, some studies do suggest that in due course of evolution, microbes can develop resistance to AMPs without added fitness cost and AMP resistant strains may even have competitive advantage over wild-type strains (Pränting et al. 2008; Lofton et al. 2013; Andersson et al. 2016). These possibilities may hinder the therapeutic application of AMPs against the microbes.

**Clinical Relevance of AMPs**

AMPs as the name suggest act as antibiotics killing the microorganisms. Interestingly, due to AMPs unique modus operandi, microbes rarely develop resistance against them. Thus, AMPs can be developed as antibiotic drugs without the problem of antibiotic resistance like the conventional antibiotics (Gallo et al. 2002). AMPs play a regulatory role in various autoimmune and inflammatory disorders, i.e., over-expression of AMPs is associated with dermatological conditions, whereas it is under-expressed in atopic dermatitis which increases infection (Pinheiro Da Silva and MacHado 2012). Use of AMPs as therapeutic in treatment of infected diabetic foot ulcers, oral mucositis, meningococcal meningitis, catheter infections, acne, cardiac ischemia, and fungal infections is currently under research (Gallo et al. 2002). Research suggests that the LL-37 AMP suppresses endotoxin-mediated lethality in rats and mice deficient in Cathelicidin-related Antimicrobial Peptide (CRAMP) and defensin are more susceptible to streptococcal and staphylococcal infections (Pinheiro Da Silva and MacHado 2012). The induction of AMPs (cathelicidin) by the active form of Vitamin D has shown role in cancer progression (Chen et al. 2018). However, the expression of LL-37 AMP shows contrasting effects with both pro-tumorigenic and anti-cancer effects. The expression of LL-37 is up-regulated in human breast cancer, and it promotes tumor growth in lung cancer, whereas LL-37 levels negatively regulate growth of gastric cancers. These findings indicate the probable role of AMPs in treatment of cancer. AMPs can also act as diagnostic markers for detection of various disorder (Pinheiro Da Silva and MacHado 2012).

Only a few AMPs have been approved for clinical usage to date, including polymyxins, which were first introduced in the 1950s (Falagas and Kasiakou 2005; Zavascki et al. 2007; Landman et al. 2008). Polymyxins are administered as topical treatment in the prevention and treatment of local infections. Gram-negative infections caused by drug-resistant microorganisms often require their use as last alternative for intravenous medications (Zavascki et al. 2007). A variety of AMPs are being developed for the treatment of diverse bacterial infections, including pexiganan and omiganan, produced from animal immunologi- cal components, and synthetic LTX-109, respectively. Two of the phase III clinical studies, confirmed that Pexiganan can treat bacterial infections associated with diabetic foot ulcers (NCT00563394, NCT00563433) (Lamb and Wiseman 1998; Lipsky et al. 2008), The topical gel for catheter infections (NCT00231153) and rosacea (NCT01784133) has been tested as a derivative of indolicidin, a bovine
neutrophil-derived AMP. LTX-109 is a synthetic antimicrobial peptidomimetic that has been tested for local use in moderate Gram-positive skin infections (NCT01223222), impetigo (NCT01803035), and in those with Staphylococcus aureus (S. aureus) nasal colonisation (NCT01158235) (Nilsson et al. 2015). The majority of AMPs such as pexiganan, omiganan, and LTX-109, are designed for indigenous use and a very few are designed for systemic delivery. Additionally, hLF1-11 known as the cationic fraction of human lactoferricin is used as intravenous formulation to treat bacterial and fungal infections in immuno-compromised stem cell transplant recipients (NCT00509938) (Van der Velden et al. 2009). In addition to hLF1-11, a variety of other AMPs are under development for treatment of fungal diseases (Table 3). For example, novexatin, a highly cationic peptide generated from defensins, is used in the treatment of obstinate fungal infections in toes, while CZEN-002, a dimeric peptide extracted from melanocyte-stimulating hormone (MSH), is used to get relief from vaginal candidiasis.

### Development of AMPs: Challenges and Clinical Applications

Despite the increasing number of clinical trials on AMPs, there is still lack of literature and studies highlighting the potential use of AMPs as therapeutic agents (Kosikowska and Lesner 2016). Till date, only seven AMPs have been approved by Food and Drug Administration (FDA) for therapeutic uses, though more than 3000 have been discovered. These findings indicate that development and use of AMPs as therapeutic agents is a cumbersome task full of challenges (Chen and Lu 2020).

The screening of identified clusters or expected peptide sequences for antimicrobial and/or fungicidal properties is the first step in the design and optimization of therapeutic AMPs for the treatment of infectious diseases using standard minimal inhibitory concentration (MIC) or minimal microbiocidal concentration (MMC) assays (Fjell et al. 2012). However, the anti-fungal and antibacterial activity of AMPs vary with varying environmental conditions making it difficult for researchers to corroborate their in-vivo findings against in-vitro results. This fact poses a major challenge in the use of AMPs as therapeutic agent. The most successful formulation of AMPs are the topical ones as the metabolic stability of AMPs is fairly low, and peptide drugs show low oral bioavailability rendering the oral formulation of AMPs highly difficult (Vlieghe et al. 2010). In addition, the intravenous formulation also suffers from short half-life due to degradation by proteolytic enzymes in blood and liver. Therefore, there is a dire need to enhance the stability of AMPs for their wide use in pharmaceutical companies. Another major drawback in large-scale production of AMPs is the high cost of production which makes it difficult to scale-up the products and market them. Further, AMPs development suffers from lack of toxicology studies and may even face regulatory hindrances due to less clinical data (Vlieghe et al. 2010). Thus,

### Table 3 Synthetic peptides effective against mycobacteria

| Peptide               | Mechanism/ Antimicrobial activity                                           |
|-----------------------|-----------------------------------------------------------------------------|
| 1-C13                 | Formation of bacterial pores (Kapoor et al. 2011)                           |
| A18G5, A24C1ac, A29C5FA, A38A1guan | Protein synthesis and bacterial membrane permeation of (Kapoor et al. 2011) |
| CAMP/PL-D             | Formation of bacterial pores (Ramón-García et al. 2013)                     |
| CP26                  | Disruption of bacterial cell wall (Rivas-Santiago et al. 2013a, b)          |
| D-LAK 120             | Inhibition of protein synthesis and bacterial pore formation (Lan et al. 2014) |
| D-LL37                | Immunomodulatory activity and formation of pores (Kapoor et al. 2011)       |
| E2 and E6             | Disruption of bacterial cell wall (Rivas-Santiago et al. 2013a, b)          |
| HHC -10               | Lysis of bacterial cell membrane (Llamas-González et al. 2013; Cherkasov et al. 2009) |
| hLFcin1-11/hLFcin17-30| Lysis of bacterial cell wall and membrane (Silva et al. 2016a, b)            |
| Innate Defense regulators (IDR)-1002, -HH2, IDR-1018 | Anti-inflammatory and Immunomodulatory activity (Mansour et al. 2015; Rivas-Santiago et al. 2013a) |
| LLAP                  | ATPase Inhibition (Chingaté et al. 2015)                                   |
| LLKKK18               | Immunomodulatory activity and formation of bacterial pores (Silva et al. 2016a, b) |
| MUI140                | Cell wall synthesis and Inhibition (Ghobrial et al. 2010)                   |
| MIAP                  | ATPase Inhibition (Santos et al. 2012)                                     |
| Pandinlin 2 (pin2) variants | Disruption of bacterial membrane (Rodriguez et al. 2014)                 |
| RN3, RN6, RN7 (1–45)  | Cell agglutination, disruption of bacterial cell wall and intracellular macrophage killing (Pulido et al. 2013) |
| Synthetic AMPs (SAMPs- Dma) | Cell penetration and DNA binding/synthetic antimicrobial peptide (Sharma et al. 2015) |
| XI(LLKK)2X; II-D, II-Orn,II-Dab and II-Dap | Formation of bacterial pores (Khara et al. 2016) |

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AMPs development and production needs much research and studies in order to be used as an alternative to regular antibiotics.

**Conclusion and Future Prospective**

Antibiotic resistance as we all know has boggled the scientific community to find alternative solutions. Antimicrobial peptides and inability of most microorganisms to develop resistance against them, shows hope in the future for the use of AMPs as therapeutic agent. The ever-rising cases of tuberculosis with less control measures urges the medical community to find innovative and sustainable ways for using AMPs’ therapeutic potential in treatment and cure of the deadly diseases. Based on review reports, antimicrobial host defense peptides have been proven in various investigations to act against *mycobacterium tuberculosis*. Natural peptides, as discussed in this paper, are amazing scaffolds for future drug discoveries due to their distinct structural topologies. Exploring the relationship between peptide structure, function as well as the molecular mechanism of action can provide a more thorough understanding of the peptides. This information will be helpful in the development of new molecules with desired properties.

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