Antimicrobial peptides and infectious diseases

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Antimicrobial peptides (AMPs) are genetically encoded, evolutionarily ancient defence molecule, which act as primary effectors of the innate immune system. Although their origin dates back to the 1920s, research on AMPs has gained momentum relatively recently after the discovery of the underlying mechanisms of innate immunity and their modulation by AMPs. In mammals, the source of AMPs is mainly the circulating and tissue phagocytes and epithelial cells, which are engaged in the killing of the engulfed pathogens and preventing tissue colonization, respectively. Direct microbicidal function of AMPs *in vitro* encompasses a broad range of Gram-positive and Gram-negative bacteria including multidrug resistant organisms, fungi, viruses and protozoal parasites. Their unique mechanism of action minimises the risk of drug resistance, hence their potential as alternatives or adjuncts to classical antibiotics is under active investigation. Apart from the microbicidal activities, multiple immunomodulatory properties, such chemotaxis, activation of immature dendritic cells, lipopolysaccharide blockage, angiogenesis and cytokine induction are critical attributes to AMPs and believed to be the principal modes of action of AMPs in infectious diseases.

A large number of AMPs grouped into several families have been reported in multicellular organisms. In humans, defensins, cathelicidins are the major categories, while others like granulysin, hepcidin and lactoferrin are also present. AMPs are cationic molecules with considerable structural diversity, based on which new synthetic peptides have been designed with the principle in mind that they exert broad-spectrum antimicrobial activities in vitroand low cytotoxicities towards host cells. The designing is centred on rational manipulations of stretches and modifications, which make them functionally more suitable for specific diseased condition or target.

In this book chapter, we discuss the major progrees made in the field of human defensins and cathelicidins in the context of infectious diseases, with special emphasis on the experimental animal models and human studies where AMPs have justified their relevance. Recent observations suggest that defects in the expression or function of specific AMPs may explain the fundamental aspects of pathophysiology of human diseases. We have selected some of the notorious gram positive and gram negative pathogens and viruses and discussed how AMPs inhibit colonization by pathogens and contribute to clearance of infections. The other major highlights of this chapter are the intricate ways that these pathogens have devised to resist the action of AMP. This is considered an important virulence mechanism of the pathogens and is critical for their survival in the host. Detailed understanding of these mechanisms will help to discover new therapeutic strategies. We have also summarized how synthetic mimics of natural AMPs have raised hope for the treatment of difficult-to-treat infections, including those due to drug resistant pathogens and have specifically mentioned those which have shown promise in the preclinical and clinical trials. Thus, in deciphering the complex scenario of host-pathogen interactions, we brought under the same umbrella the progress made in the field of natural and synthetic AMPs to combat pathogenic infections and their tactics to evade these host innate defences in order to reflect how these agents can be manipulated therapeutically in favour of the host and may be an alternative to the currently available antibiotics.

Keywords: AMPs; HDPs; Defensins; Cathelicidins; Synthetic AMPs; Infections; Gram positive; Gram negative

1. Introduction

Mammals continuously protect themselves from harmful microorganisms. The most common site of invasion are the epithelial surfaces like gastrointestinal tract(GI tract), respiratory tract, moist surfaces of eyes, skin, nose, oral cavity etc. Encounter with a pathogenic microbes initiates immunologic response of two types; innate immune response and adaptive immune response. The second set of response generates antigen specific response, and takes days to build, so the first set of response is the initial barrier. Innate immune system is the first line of defense which is non-specific in nature and is induced rapidly. It operates through number of immune and non-immune cells. The immune cells include neutrophils, dendritic cells, macrophages and the non-immune component involves epithelial surfaces which are the primary site of interaction with external environment and handles numerous microorganisms. The effector arm of innate immune system has two branches; pathogen recognition receptors (PRRs) and antimicrobial peptides (AMPs). PRRs recognize conserved pathogen associated molecular patterns (PAMPs). PAMPs when bind to PRRs of pathogenic microbes, initiates an inflammatory signaling cascade leading to expression of various proinflammatory molecules like cytokines, chemokines and antimicrobial responses, thus eliciting the primary response to infectious pathogens. Innate immunity in addition also shapes adaptive immune response. The antimicrobial function of the innate immune response is partially carried out by AMPs which are capable of directly killing microbes.

AMPs are evolutionarily conserved molecule starting from single cell microorganisms to multicellular insects, plants, invertebrates, mammals etc. They are gene encoded cationic molecules which are less than 12-50 amino acids, amphipathic in character with broad spectrum of activity including bacteria, viruses, fungi, protozoa etc. Antimicrobial peptide database (APD) has 2578 AMPs (last updated on 11th June, 2015) including 257 bacteriocins from bacteria, 2 from archaea, 7 from protists, 13 from fungi, 319 from plants and 1939 from animals.

Infectious diseases are one of the major crisis worldwide with potential life threat. Treatments of infectious diseases are becoming cumbersome task due to the emergence of antibiotic/multidrug resistant (MDR) pathogenic strains. Thus AMPs with unique mode of action, pleiotropic function, and ubiquitous presence has fetched considerable attention in exploring their basic biology, structure, regulation as well as preferential use as 'next generation antibiotics' to treat infectious diseases.

In this book chapter we synopsize overview of AMPs which are abundantly expressed in humans, their mode of action, and role in infection especially focusing on major Gram positive and Gram negative pathogens which are poses potential threat. We also discuss in this relevance how microorganisms modulate their expression to their own benefit. Here we try to revisit the topic how synthetic AMPs, based on natural AMPs, have been recently employed in treating different infections. As in case of natural antibiotics, AMP resistance although rare, "is still not that rare" incidence as microorganism has managed to acquire some strategies to avoid the activity of AMPs also.

2. Overview of natural AMPs and their mode of action

AMPs are also popularly named as host defense peptides (HDPs). Expression of AMPs may be constitutive or inducible, the latter being in response to microbial challenges and proinflammatory stimuli. AMPs have direct microbicidal potential rendered by their net positive charge, allowing them to interact with the anionic microbial membranes. In physiological conditions, however, they cannot exert their direct antimicrobial functions due to the low local peptide concentrations and presence of multiple proteases, divalent cations, anionic glycosaminoglycans etc. Thus, the other mode of action of AMPs, the immunomodulatory arm is more relevant in fending off infectious microorganisms. AMPs can stimulate production of cytokines and chemokines, augment prostagladlin release, increase phagocytosis and recruit inflammatory cells at the sites of infection, neutralise LPS, promote wound repair, chemotaxis and angiogenesis etc. Based on the structure, AMPs are categorised into different families, cathelicidins and defensins being the major families in the humans.

2.1 Defensins

Defensins are 30 amino acids long and are divided into three subfamilies, α, β and θdefensins. α- and β-defensins have three antiparallel β sheet motifs with 6 cysteine residues connected by three disulfide linkages, although their positions differ in the two families. However, θ-defensins which are small and circular peptides stabilised by disulfide bonds are absent in humans, but found in the rhesus monkeys. α- defensins are of two main types; those present in the neutrophil granules are called human neutrophil peptides (HNP-1, HNP-2, HNP-3, and HNP-4), while HD-5 and HD-6 are secreted by the Paneth cells of the small intestine. In humans, the major functional β-defensins are HBD-1, HBD-2 and HBD-3. The source of expression of different AMPs with their spectrum of activity is tabulated in Table 1. Some of the β-defensins like HBD-1 are constitutively expressed, while others are inducible by various stimuli [1, 2].

2.2 Cathelicidins

Cathelicidins, the name proposed by Zanetti et al in 1995, are the class of AMPs, which bear the signature 'cathelin' domain at their N terminus and cationic antimicrobial domain at the C terminus. The cathelin domain retains high sequence homology across the species, while the antimicrobial domain shows significant sequence diversity. The cathelin domain is approximately 100 amino acids long, and flanked by a signal peptide domain of 30 amino acids and an antimicrobial domain of 12-100 residues. Cathelicidins are present in the cell cytoplasm as precursor molecules called the 'preprotein'. The cleavage of the cathelin domain releases the mature cathelicidin-derived AMP, which is the active functional form. Humans have only one cathelicidin called LL-37. Its immunomodulatory functions include anti-endotoxin activity, mast cell degranulation, angiogenesis, skin re-epithelialisation, chemotaxisetc[3][4]. LL-37 expression sites as well as its antimicrobial spectrum are tabulated in Table 1.

Table 1

AMP	Subgroups	Sources of expression	Spectrum of activity	Reference
Defensins	HNP-1	Majorly expressed in granules of neutrophils, NK cells but also found in T cells, B cells, monocytes/macrophages, immature DCs, bone marrow, respiratory tract	*S. aureus*, *P. aeruginosa* , *E.coli* , HIV	[1, 2]
α-defensins	HNP-2			
	HNP-3			
	HD-5	Paneth cells of GI tract. HD5 is also found in kidney and in male and female reproductive tract	HD5 (*L. monocytogenes, E. coli* , *S.* Typhimurium, *C. albicans, C. difficile,* HPV, influenza virus, HIV)	[5-7]
	HD-6			

β-defensins	HBD-1	Human plasma, GI tract, airway and genitourinary epithelium, organs like kidney, prostrate, testis vagina, uterus and thymus	*C. albicans*, *B. fragilis*, *E. faecalis* and *E. coli*	[1, 2]
	HBD-2	Intestine, trachea, oral and nasal mucosa ,skin, eyes, salivary glands, urinary tract, monocytes, macrophages, dendritic cells, NK cells and T-cell	*P. aeruginosa*, *E.coli* and *C. albicans*,but bacteriostatic towards *S. aureus*	
	HBD-3		*E. coli, S. aureus, P. aeruginosa, S. pyogenes, E. faecium*vancomycin resistant *P. gingivalis*, *C. albicansetc*	
Cathelicidins	LL-37	small intestine, differentiated colon, keratinocytes, airway,myeloid cells ,bone marrow, thymus, liver, spleen and pancreas	*E. coli, K. pneumonia*, *P. aeruginosa*, *N. gonorrhoeae* ,GAS*, H. pylori, Shigellasp, Salmonella sp*and *C. albicans*, *S.mutans, P. gingivalis*, and *A. actinomycetemcomitan,* parasite and enveloped virus.	[8, 9]

2.3 Synthetic Peptides

AMPs isolated from the natural source are less than ideal targets as "next generation antibiotics" to deal with the MDR microbial pathogens, because of issues like salt and protease sensitivity, cytotoxicity, poor bioavailability etc. However the unique physicochemical properties of the AMPs inspired the designing of synthetic mimics of natural AMPs with various alterations incorporated into their structures. This retains the critical properties of AMPs, while eliminating the disadvantageous traits, which make them imperfect as therapeutic drugs. Understanding of the 'structure-activity' relationships of AMPs will make designing of synthetic compounds easier. The modifications incorporated in designing these peptides are based on the physicochemical properties like the charge, amphipathicity, solubility, length, helicity, active domain etc. Minimal alteration of any of these features may come up with peptides having a different function and spectrum. Computer-assisted designing of synthetic peptides and prediction of functions is another very productive and less tedious strategy towards the discovery of new AMP-based drugs. Other strategies include incorporation or deletion of amino acid residues (congeners) [10, 11], synthesis of hybrid AMPs [12, 13], conjugated peptides-AMPs tagged with either some ligand or some antibody which can specifically bind to the target pathogen surface receptor [14], immobilised peptides where AMPs are efficiently immobilised on the matrix of different materials retaining their antimicrobial functions [15], cyclotides-circularised stable peptides that are protease resistant [16], variants with reduced cytoxicities, hemolytic activities, functionally more active in complex biological fluids etc. Synthetic AMPs are of several types; those with both immunomodulatory and anti-infective properties; with only immunomodulatory and no anti-infective property and anti-infective peptide with no immunomodulatory functions[17]. Synthetic AMP designing has taken a leap forward with peptidomimetic studies, an approach that helps in identifying biologically and physiologically active AMPs from the vast ocean of peptides present in the nature [18]. Synthetic AMPs with majorly immunomodulatory function are also known as innate defence regulators (IDRs). Table 2 tabulates different synthetic AMPs, which are important with respect to clinical trials and preclinical testing in the field of infectious diseases.

Table 2

Synthetic AMP	Parent AMP/source	Spectrum/Activity	Comments	References
Brilacidin/PMX-30063 (PolyMedix)	aromatic synthetic peptide, defensin mimetic	*S. aureus* and MRSA	Phase IIb for acute Bacterial Skin and Skin Structure Infections (ABSSSI) by *S.aureus*	"ClinicalTrials NCT020388" [19]
SMAMP02	synthetic antimicrobial oligomers (SMAMPs)	*S. aureus*	Preclinical trials: neutropenic mouse model of *S. aureus* infection	[20, 21]
P.60.4Ac , P10	derivatives of natural AMP	methicillin resistant *S.*	attractive therapeutic	[22]

	LL37	*aureus*(MRSA LuH14616) and mupirocin-resistant MRSA strain (LUH15051)	agents to treat burn wounds infected with multidrug resistant bacteria	
IK8L	synthetic AMPs with β pleated sheet structure and sequence	MDR strains of *P. aeruginosa, A. baumannii, M. tuberculosis* , *S. aureus, K. pneumoniae* (interferes with biofilm formation)	Still in intial phase of research	[23]
Pexiganan acetate (MSI-78)	Manganin derivative	Antinfective, treatment of diabetic foot ulcers, used as topical antibiotic	Phase IIII ; Failed approval of US Food and Drug Administrati on (FDA) for medical use	[24]
Omigananpentahydrochol oride	Indolicidin derivative	Bactericidal, antifungal, catheter assosciated infections Papulopustular Rosacea	Phase IIII b In Phase IIII	(ClinicalTrial.http://clinicaltrials.gov/show/NCT 017841) [25, 26]
Iseganan (IB-367)	protegrin-1 derrivative	In treatment of oral mucositis	phase III initiated for oral mucositis in patients receiving radiation therapy for head-and-neck malignancy	[27]
rBPI21	BPI derivative	Meningitis	Phase IIIb	[28]
hLF1-11 (AM Pharma)	derivative of human lactoferrin	Gram-positive and Gram-negative bacteria, including MRSA and MDR *A. baumannii*, fluconazole-resistant *C. albicans*	Fungal and bacterial infections that develop during neutropenia in haematopoie tic stem cell transplants (HSCT); Phase I/ II	https://clinicaltrials.gov/ct2/show/NCT00430469 [29]
P-113 (Demgen, Pittsburgh) Zn(II)-P-113:metal bound form	peptide is derived from the 12 residues of histatin 5	*C. albicans*, *C. glabrate*, *C. parapsilosis*, and *C. tropocalis*,along with fluconazole resistant *C. albicans and C. glabrata* *S. aureus*, *E. faecalis*, and *C.*	Approval by Investigation al New Drug for oral candidiasis Phase IIb for HIV patients, and another variant, P113D is	ClinicalTrials.gov identifier: NCT00659971[30-32]

		albicans	under consideration treatment of *P. aeruginosa* infections in cystic fibrosis patients	
CZEN-002	α-MSH derivative designed based on the KPV (α-MSH11-13) peptide	*C. krusei* and *C. glabrata*, whichare emerging as drug-resistant strain	Phase IIb, in treatment of vulvovaginal candidiasis	[33-35]
Ceraginins are cationic steroid antibiotics (CSAs) CSA-13 Other CSAs, CSA-19,-131including CSA-13	mimic host defense peptides Lead ceraginin	multidrug resistant organisms such as *Pseudomonas*, MRSA, and VRSA, Candida sp, orthopox virus. Bactericidal against LESB58, a hypervirulent strain of *P. aeruginosa*.	squalamine cationic steroid antibiotic for topical and systemic use, anti-biofilm activity against *P. aeruginosa*, CSA-13 and CSA-131 showed anti-biofilm activity against LESB58 and their activity is retained in the CF sputum	[36-38]
IMX942	A derivative of IDR-1	*S. pneumoniae*,MRSA and vancomycin-resistant *Enterococcus sp*	phase II for attenuation of infections associated with cancer chemotherapy.	[39]
HB1345 and HB1275 (Helix BioMedix)	lipohexapeptides	*S. aureus*, *Streptococcus pyogenes*, and *P. aeruginosa*, *Candida* and *Trichophyton* sp, MRSA and Vancomycin resistant *Enterococci*.	deliver clinical benefits comparable to Terbinafine, a drug approved by the FDA for onchomycosis; Preclinical.	[40]
RC-101	Recreated θ-defensin	Anti-HIV.	potential as topical microbicide for HIV-1	[41]
IDR-HH2 and IDR-1018	Derivative of bovine bactenecin (Bac2A)	drug-sensitiveand multidrug-resistant *M. tuberculosis*	Initial phase of research	[42]

2.4 Generalized mode of action of AMPs

The basic and classical mode of action of AMPs involves their direct action on microbial membrane causing cell membrane damage. The positively charged amino acids of cationic AMPs interact electrostatically with negatively charged residues of microbial membrane. Composition of the microbial cell membrane thus determines the specificity of action of AMPS. AMPs distinguish between eukaryotic and prokaryotic membrane by the lipid composition of extracellular surface. The extracellular surface of eukaryotic membranes is enriched with neutral phospholipids like sphingomyelin and phospatidylcholine (PC) whereas prokaryotic cell membranes are made up of anionic phospholipids like cardiolipin and zwitterinic phospholipids like phospatidylethanolamine (PE). Again the outer membrane of Gram negative bacteria have LPS as extracellular structure and gram positive cell wall have teichoic and techiuoronic acids. These molecules which impart negativity of bacterial membrane are essential targets of AMPS. The secondary structure assumed by these cationic AMPs are also important in determining the way it inreacts with the target membrane. The AMP: lipid ratio and affinity allows it to insert into the lipid bilayer generating pores. Apart from generating pore it can give rise to other types of membrane perturbation like phase separation, disruption of membrane bilayer and formation of non-lamellar lipid structure.

There are different models which have been proposed in describing the way AMPs interacts with microbial membrane like 'barrel-stave'[43, 44]'torroidal'[45, 46], 'agreegate'[46, 47][48, 49] and 'carpet[47][50] models and the mechanism of action varies with molecular properties of AMP involved and lipid membrane in scenario. Finally, apart from AMP induced action membranolytic actions, they have additional intracellular targets like inhibition of cell wall synthesis, DNA, RNA, protein synthesis. However, single AMP can have multiple targets of action.

3. Role of natural AMPs in Gram-positive and Gram-negative infections*:*

As discussed earlier AMPs are so called novel antibiotics with broad spectrum of activity and specially effective in curing drug resistant microorganisms. There have been more than two deacdes of AMP research but any AMP is still not been able to establish the foothold of the stature of classical antibiotics and thus did not manage approval from Food and Drug Administration (FDA). In the field of infectious dieases, AMPs have considerable advantages over antibiotics in their therapeutic applications; mode of action, broad spectrum, low chances of resistance development, rapid activity and immunomodulatory arm supporting and playing a big role in augmenting its microbicidal activity. In the following section we diccuss the role of major AMPs like defensins and cathelicidins in some of the major Gram positive and negative bacterial infections.

3.1 Major Gram-positive infections

Staphylococcus aureus: *S. aureus* is a major human pathogen that infects the skin, soft tissues as well as the internal organs. Systemic infection by S. aureus is particularly common in the immunocompromised subjects. S. aureus colonizes the anterior nares of 25-50% of healthy individuals and this is considered an important risk factor for subsequent infection by the pathogen [51].Among the AMPs, LL-37 and HBD-3 have the highest activities against S. aureus[52, 53][54].Significantly higher antimicrobial activity of HBD-3 comapred to other HBDs may be explained by its ability to form amphipathic dimers at low concentrations with increased positive charges on the surface [55].Both peptides are found in the nasal secretions with*S. aureus* carriers and non-carriers having equal levels of HBD-3, while LL-37 levels are higher in the carrier state. However, the difference was lost in patients suffering from nasal polyps [56].Synergism between the antistaphylococcal activities of HBD-3 and LL-37 was found in the airways surface liquid (ASL). However, an acidic pH, as found in cystic fibrosis patients suppresses the individual as well as synergistic antimicrobial functions [57].HBD-3 can eliminate biofilm formation by methicillin resistant *S. aureus* (MRSA) (ATCC43300) through the regulation of icaA and dltB [58, 59]. Other AMPs like HBD-1 shows little antimicrobial activities against *S. aureus*, but Defb1(-/-) mice harboured significantly higher number of Staphylococci ($P = 0.008$) in the bladder compared with the controls, suggesting a role for HBD-1 in the resistance to urinary tract infection [60].Another study found an association between HBD-1 promoter polymorphism, leading to decreased beta-defensin secretion and persistent nasal carriage of *S. aureus* [61]. Increased nasal colonization is seen in WG patients and nasal epithelial cells (NECs) from these individuals induce lower levels of HBD-3 compared to healthy subjects [62]. In the skin, HBD-2,-3 and LL-37 are induced in the keratinocytes by live- or heat-killed *S. aureus*and its LPS and LTA [53, 63-67]. *S. aureus* is reported to regulate HBD-3 expression through the activation of TLR2-p38MAPK-AP-1 signaling pathways [65]. HBD-3 induction is significantly lower in more severe and recurrent skin infections in a study involving 32 patients [67].In atopic dermatitis (AD) patients, Th2 cytokines suppress HBD-3 induction by *S. aureus*[68].Epidermal growth factor receptor (EGFR) signalling activation through wounding induces HBD-3 and thus augments *S. aureus* killing [69].However, a recent report suggests that EGFR inhibitors suppress HBD induction by *Staphylococcus epidermidis* (*S. epidermidis*), but not *S. aureus* [70]. Exopolysaccharide intercellular adhesin (PIA) is a major biofilm matrix for *S. epidermidis*, and a PIA mutant strain is significantly more susceptible to killing by the skin antibacterial peptides like HBD-3, LL-37 and dermcidin. Interestingly, defective β-defensin expression in the human skin is associated with persistent nasal carriage of *S. aureus* [61]. Cathelicidins are also effective against *S. aureus* in

the keratinocytes [71-73]. A recent report in the journal of 'Science' showed that adipocytes derived from cathelicidin knockout mice loses the capacity to inhibit *S. aureus* infection [74]. Vitamin D is a major regulator of LL-37 expression [75-77]and children with vitamin D deficiency suffer from recurrent skin and soft tissue infection due to *S. aureus* [78]. LL-37 effectively killed extracellular *S. aureus* in nanomolar concentrations as opposed to millimolar concentartions of doxycycline and cefazolin. LL-37 is also superior to antibiotics in clearing intracellular bacteria [79]. It is shown that graRS regulatory genes controls D-alanylation of teichoic acid and their deletion alters the bacterial surface charge, leading to increased susceptibility to LL-37 and attenuated virulence in mice [80]. However, wall teichoic acid deficiency led to 100-fold more resistance to HBD-3, but not to other AMPs including LL-37 [81].A number of studies reported thatMRSA strains showed significantly increased resistance to LL-37-mediated [82, 83]. Among the α-defensins, HNP2 is more effective than other HNPs against *S. aureus* [84, 85].HNPs are abundantly expressed in the lesions of superficial folliculitis of the skin caused by *S. aureus* infection [86]. The underlying mechanism of antimicrobial action of HNP-3 involves binding to the LukS and LukF components of Panton-Valentine leukocidin (PVL), a pore-forming toxin of *S. aureus*, inhibiting neutrophil lysis by the toxin [87].Another AMP RNAse7 also has *S. aureus* in its spectrum of activity [88]. *S. aureus* also managed to design various strategies to evade these AMPs and survive colonization. For example, it destroys the AMPs like LL-37 and dermicidin by secreting proteases like aureolysin[89, 90]produces AMP binding molecules like fibrinolytic enzyme staphylokinase [91], modifies teichoic acid using D-alanine or phospholipids with L-lysine thus reducing the net negative charge of bacterial cell surface [92, 93] and alteration of bacterial cell surface hydrophobicity [94, 95]. Community acquired MRSA strain USA300 prevents modulation of Staphylococcal protein A by AMPs (HNPs) and antibiotics (clindamycin) unlike in other community acquired-MRSA strains. This justifies the virulence and successful spread of USA300 strains [96].Synthetic peptides which are in limelight as therapeutics to treat *S. aureus* and its antibiotic resistant strains are quite a few and are tabulated in Table 2.

Mycobacterium tuberculosis: Tuberculosis (TB) is a worldwide health problem. While no new drugs are developed for first line antitubercular therapy in the last 40 years, the problem of multidrug resistance (MDR) and extensive drug resistant (XDR) strains have gripped many countries in the world. AMPs have emerged as attractive new candidates for therapy and HNPs are particularly significant in this regard. HNP-1 is reported to kill highly virulent *M. tuberculosis*strain (H37Rv) in vitro as well as ex vivo at the dose of 40 μg/ml, and approximately 98% of intracellular bacteria are eliminated within 3days of treatment [97]. However, another study found that HNPs failed to kill a different strain of *M. tuberculosis* in vitro even at a much higher concentration (250μg/ml), although mediated clearance of the intracellular bacteria from healthy neutrophils within an hour [98]. In a study involving 189 adults exposed to patients suffering from active pulmonary tuberculosis, risk of infection is inversely proportional to the peripheral blood neutrophil counts [99]. Mouse models further suggested therapeutic potential of HNP-1 against experimental tuberculosis. Subcutaneous administration of HNP-1 reduced the bacillary load from the lungs, liver and spleen in a concentration and time dependant manner. Within one week of therapy, there is a significant decrease in the CFU counts in the treated mice that reached one log difference compared to the control mice within a period of 2-4 weeks [100].HNP-1 acts synergistically with isoniazid and rifampicin to kill intracellular mycobacteria. Treatment of BALB/c mice with the combined regime for four weeks cleared mycobacteria from the visceral organs much more efficiently than treatment, which did not incorporate HNP-1 [101]. While mycobacterial cell wall/membrane is the major target(s) of HNP-1[102], it could directly inhibit DNA biosynthesis [103]. Intra-tracheal administration of HNP-1, alone or in conjugation with HBD-2 and in the presence of L-isoleucine prevents the transmission of tuberculosis and reduces the bacterial load and pulmonary lesions [104]. The chemotactic effects of HNPs released by neutrophils may drag monocytes to the site of lesion and macrophages derived from these cells may take up extracellular HNPs in vivo to kill the intracellular pathogens [105]. A recent study showed close association between MDR-TB with low plasma concentrations of HNP1-3 [106]. Published study suggested that a heterologous tandem peptide, 'HBD-3-M-HBD-2' could inhibit multidrug resistant strain of *M. tuberculosis* [107]. Human alveolar macrophages either do not express α and β defensins or express HBD-2 only after a high MOI of infection with *M. tuberculosis.* This is in contrast to macrophages from other species and explains, at least partly, the host restriction of *M. tuberculosis*. This is supported by the mycobacteriostatic and mycobactericidal activities of human macrophages transfected with HBD-2 mRNA [108]. In contrast, human lung epithelial cell line A549 after *M. tuberculosis* H37Rv or *M. bovis* BCG infection readily induces HBD-2 mRNA expression [109]. The BCG effects are dependent on PKC and Ca+2-Calmodulin pathways and mediated through the activation of JNK, p38 MAPK and PI-3K [110, 111].A 18-30 kDa cell wall protein of BCG was reported to stimulate HBD-1 mRNA expression in the pulmonary glandular epithelial cells by inducing binding of C/EBP beta, AP-1 and CP2 in the upstream regulatory region [112].It is also reported that particulate matters of 2.5 and 10 μm suppress HBD-2 and -3 production by A549 cells following *M. tuberculosis* infection and increase the intracellular growth [113]. Two DNA vaccines were prepared by the fusion of HBD-2 and the immunodominant mycobacterial antigens, ESAT6 (pDE) and 85B (pDA). BalB/C mice prime boosted with BCG followed by the DNA vaccines showed less tissue damage and significantly higher survival compared with the BCG alone when challenged with the H37Rv strain or highly virulent clinical isolate LAM 5186 [114].Cathelicidins have antimycobacterial effects with minimum inhibitory concentrations ranging from 2 to 10 μg/mL. In the *M. tuberculosis* lung infection model with the drug-sensitive H37Rv strain or an MDR strain, intratracheal administration of LL-37 (1mg/kg) three times a week

resulted in significant reduction of the bacillary load in the lung within 1 month [115]. LL-37 added from outside or overexpressed within macrophages significantly reduced the intracellular survival of mycobacteria. Mycobacterial killing is significantly impaired in the CAMP knock-down macrophages and *camp(-/-)* bone marrow-derived macrophages [116]. During *M. tuberculosis* infection, TLR1/2 is activated on the circulating macrophages, which result in induction of Vitamin D receptor (VDR). VDR along with 1,25-D3 induces LL-37 to check the bacterial growth [117, 118].A positive correlation ($p < 0.05$) is observed between macrophage phagocytosis and LL-37 expression in peripheral blood mononuclear cells (PBMCs) from 50 healthy controls and 35 pulmonary tuberculosis patients cultured with *M. tuberculosis* with or without 1,25(OH)2D3. Moreover, LL-37 induction by 1,25(OH)2D3 is more prominent in the cavitary lung disease [119]. In a recently published study, LL-37 mediated autophagy induction by 1,25-D3 through increased transactivation of the autophagy-related genes Beclin-1 and Atg5. Autophagy and LL-37 are required for the antimycobacterial activities of the physiological concentartions of 1,25-D3 [120].TLR stimulation of macrophages also induces vitamin D-1-hydroxylase genes, leading to cathelicidin upregulation, which clears intracellular *M. tuberculosis* [75]. In this study, African-Americans were found to suffer from vitamin D deficiency, impaired cathelicidin induction and increased susceptibility to tuberculosis. However, other reports failed to correlate Vit D and LL-37 levels in the serum before and after supplementation [121, 122].An attempt to use LL-37 as a biomarker for TBM showed a cut-off value of 3221.01 pg/mL in the CSF of a cohort of 56 patients with a sensitivity of 0.52 and a specificity of 0.95.9 [123]. Comparison of *M. tuberculosis* H37Rv infection of A549 epithelial cells, alveolar macrophages (AM), neutrophils and monocyte-derived macrophages (MDM) showed maximum LL-37 induction in AM. However, LL-37 is not present in tuberculous granulomas, suggesting that its role may be restricted to early infections [124]. Treatment of alveolar macrophages with the NOD2 ligand MDP improved the control of virulent *M. tuberculosis* through the release of TNF-αand IL-6 and overexpression of LL-37 [125]. *M. bovis* BCG-mediated up-regulation of LL-37 in A549 cells is found to be mediated through MEK1/2, p38 MAPK and NADPH/ROS signaling pathways [126, 127].). ROS also regulated LL-37 expression in the keratinocytes infected with *M. ulcerans* through the activation of TLR2, Dectin-1 and TLR4, and suppression of ROS and LL-37 markedly enhanced intracellular *M. ulcerans* growth in the cells [128].

IDRs are reported to be effective against *M. tuberculosis* infections and showed therapeutic potential. IDR-HH2 and IDR-1018, but not IDR-1002, reduce bacillary loads in mouse models of drug-sensitiveand multidrug-resistant *M. tuberculosis* infections, despite havingonly modest *in vitro* anti-mycobacterial activity. An immunomodulatory peptide currently in use as an anti-infective agent is the drug glutoxim (NOV-002). This is prescribed in Russia as an adjunct to traditional therapies for the treatment of pulmonary and disseminated Mycobacterium tuberculosis infections [129]. Synthetic AMP administration may function as vaccine adjuvants. Mice immunized with a combined DNA vaccine along with $KLKL_5KLK$ peptide conferred significantly higher protection against virulent *M. tuberculosis* strain than the combined DNA vaccine alone or BCG immunization. This was achieved by increased production of IL-12 and IFN-γ and enhanced antigen-specific cytotoxic T lymphocyte activity[130].

Clostridium difficile:Clostridium difficile (*C. difficile*) infection is an increasing health problem, particularly of the industrialized world. The disease spectrum includes mild to moderate diarrhea to severe pseudomembranous colitis. Infection is transmitted mainly by the spores, which are resistant to most antibiotics and when the drugs are effective, recurrence rates are very high. Most AMPs are active against this gastrointestinal offender with varying degrees of efficacy. HD-5 is the most abundant AMP in the human intestine. A recently published study has found that HD-5 kills the most prevalent hypervirulent strains of *C. diificile* in the European population at physiological concentrations, but spares the commensal *E. coli* and Enterococci. The effect is mediated through membrane depolarization and bacterial fragmentation[131]. Other α-defensins (HNP-1 and HNP-3) were reported to bind selectively to toxin B of *C. difficile* in vitro and neutralise its effects on glucosylation of Rho guanosine triphosphatases [132].While LL-37 is ineffective against toxin B, exogenous LL-37 suppresses intestinal inflammation and tissue damage in mice infecetd with C. difficile as well as toxin A-mediated inflammation in the ileal loops [133].A study evaluated various combinations of AMPs and antibiotics against both toxin-secreting and non-secreting strains of *C. diificile.* HBD-3 and LL-37 synergised with various antibiotics, such as tigecycline, moxifloxacin, piperacillin-tazobactam and meropenem in bacterial killing by increasing the uptake of antibiotics, perhaps through membrane damage. Some combinantions may augment toxin release, which may be neutralized by the addition of HNP-1 [134].

3.2 Major Gram-negative infections

S. Typhimurium:*Salmonella enterica*serovarTyphimurium (*S.* Typhimurium) is an enteroinvassive gram negative pathogen. It is generally transmitted due to ingestion of contaminated food and water. In human the pathogen causes self-limiting gastroenteritis and in mice it develops typhoid like fever. Most of the pathogen is cleared off but that which survives the stomach acidic environment enters into small intestine and breaches the epithelial barrier. When the barrier is breached a number of innate defences come into action which includes cationic AMPs. *S.* Typhimurium is susceptible to α-defensins, β-defensins as well as cathelicidins. The cytoplasmic receptorNOD2 has been found to play role in the clearance of *Salmonella* [135]. Yamamoto-Furusho*et .al* showed that HNP-1 secretion is stimulated by the NOD2 ligand muramyl dipeptide (MDP) in the intestinal epithelial cells and HNP-1 knockdown in the cells interferes with NOD2-dependant antibacterial functions[136]. Paneth cell defensins HD-5 and HD-6; secreted by the crypts of Lieberkühnalso regulates microbial load of the small intestine and renders protection of the neighboring stem cells at

the bottom of the crypts from the luminal pathogens [5, 6, 137, 138]. Purified HD-5 at 100 μg/ml reduces the CFU count of wild-type *S*. Typhimurium by 99% [137]. To further support this role, HD-5 transgenic mice showed protection against *S.* Typhimurium infection [139]. Another mouse model, which establishes the antibacterial role of HD-5 is the MMP-7 knockout mice that fails to clear enteric pathogens [140]. MMP-7 is essential for the processing of cryptdins (murine defensins) from their proform to the active peptides within the paneth cell granules. HD-6 is found to be protective against *S*. Typhimurium-infected mice by self-assembly after binding to the bacterial surface proteins and forming nanonets and fibrils [140-142].

Human β-defensins and cathelicidins are also active against Salmonellosis. Plant derived purified HBD-1,-2 increased survival of Salmonella-infected mice [143]. A similar report shows that the use of recombinant HBD-1 and HBD-2 at 1:1 ratio can be therapeutically effective against murine Salmonellosis [144]. HBD-2 expression is up-regulated in human fetalxenografts infected intraluminally with Salmonella [145]. In murine *S*. Typhimurium infection model, intravenous delivery of cathelicidin-expressing lentivirus ameliorates cecal inflammation and suppresses collagen synthesis [146]. Epithelial cell resistance to *S*. Typhimurium (ATCC 14028) is increased by transient transfection of LL-37 mRNAs [147]. Host neuroendocrine stress hormones can regulate *S*. Typhimurium virulence genes, virKamd mig14, which are involved in AMP resistance. Mutants of these genes succumb more easily to LL-37 activity [148]. A number of model LL-37 peptides were designed, which have no AMP activity, but retain the immunomodulatory functions and are still capable of protection against *S.* Typhimurium infection in mice [149].

S. Typhimurium has number of ways to bypass these AMPS [150]. This pathogen has several two compartment regulatory systems, such as PhoP-PhoQ and PmrA-PmrB, which help them to sense the local environment and evade the host defence by modifying gene expresion [151, 152]. Presence of AMPs serves as the stimulus for *Salmonella* and generates signals to activate PhoQ[153, 154][155].A study revealed eight important loci in the Salmonella genome which are related to AMP resistance. The analysis of the mutants showed one to have impaired LPS generation and another mutant harboured a defect in phoP[156]. Lipid A changes due to PmrA also induces AMP resistance by decreasing the net negatives charges on the outer membrane. PmrA regulates the production of aminoarabinose, which is attached to Lipid A through *pmrHFIJKLM*operon. PmrA and PmrF mutants are defective in aminoarabinose production and are more susceptible to AMP killing. These mutants are particularly defective in oral challenge. Similarly, aminoarabinose production (Ara4N) deficient mutants face survival challenge in mice [157, 158]. Another study found no role for AMPs in the increased susceptibility of Ara4N deficient *S.*Typhimurium[159]. *S.* Typhimuriumgenome has several genes called yejA, yejB, yejE and yejF lying in yejABEF operon, which code for 'ATP-binding cassette (ABC) transporter'. When the yejF gene is deleted, the pathogen gets susceptible to HBD-1 and HBD-2, and gets attenuated in their capacity to proliferate inside the macrophages. In a murine typhoid model, this mutated strain showed reduced virulence when injected intragastrically[160].*S.* Typhimurium LT2may be made AMP resistant by passing them serially through increasing concentrations of LL-37. The resistance gene is mapped by whole genome sequencing to the pmrB and phoP locus as well as LPS biosynthesis locus called waaY or rfaY[161]. This waaY mutant has reduced susceptibility to nearly all AMPs, and has the advantages over the wild type bacteria for host infection.

*Klebsiellapneumoniae:*This gram negative encapsulated nosocomial pathogen cause urinary tract infections to severe pneumonia and neonatal sepsis associated with high morbidity and mortality [162]. Klebsiella is notorius for multidrug resistance and constitute a large number of ESBL strains and the nearly untreatable carbapenem resistant pathogens However, the bacteria remains susceptible to defensins and cathelicidins. A number of ESBL-producing *K. pneumoniae* (36) and *K. oxytoca* nosocomial are reported to be sensitive to the recombinant HBD-2 and -3 [163]. Interestingly, the bactericidal activity of HBD-2 is less against the wild type strain than multi-drug resistant *K. pneumoniae,* especially the beta-lactam antibiotics resistant strains [164]. *K. pneumoniae*is a major urinary tract pathogen and the culture supernatants of the proximal and distal renal tubular epithelial cells (RTCs) shows antibacterial activity against *K. pneumoniae* due to the presence of HBD-1 and HBD-2 [165]. However, the anionic capsular polysaccharides (CPS) of *K. pneumoniae* are a major virulence factor and add to AMP resistance.*K. pneumoniae* CPS mutant is found to be resistant to HNP- 1, HBD- 1, lactoferrin, protamine sulfate and polymyxin B [166]. The resistance is lowered by previous exposure to the sub-inhibitory concentrations of different antibiotics, including ciprofloxacin, levofloxacin, nalidixic acid, which increased the permeability of the outer membrane and also enhanced its binding to AMPs [167]. *K. pneumoniae* K2-derived purified CPS enhances the resistance of un-encapsulated *K. pneumoniae* mutant to polymixin B. Anionic CPS released by the treatment of Polymyxin B and HNP-1 from the bacteria over-expressing CPS binds to the cationic AMPs, thus lowering the amounts of available AMPs to kill the bacteria [168]. In contrast to the wild type bacteria, CPS mutant *K. pneumoniae* induces HBD-2 and HBD-3 expression both in vitro and in-vivo. HBD-2 induction is mediated by TLR2 and TLR4, while NOD1 regulates HBD-3 expression [169]. In addition, Klebsiella outer membrane protein (KOmpA) activates TLR2 present on the surface of $CD56^{+}CD3^{-}$ NK cells and induces rapid release of HNP1-3 from the storage granules of these cells. On the other hand, *K. pneumoniae* endotoxin induces HBD-4 in the murine lungs in a TLR4-dependant, NF-kappa B mediated pathway [170]. In a study with three N-terminal deletion mutants of HBD-3, the peptide fragment deleted of nine N-terminal residues was found to have reduced bactericidal activity against *K. pneumoniae*. Deletion of three N-termial residues of HBD-3 resulted in significantly higher antimicrobial activity compared to the parental molecule against almost all the tested strains and at

high NaCl concentrations [171]. Murine cathelicidin CRAMP is effective against *K. pneumoniae* in the pneumonia model. CRAMP is induced by intratracheal infection of *K. pneumoniae* and CRAMP-/- mice showed poor bacterial clearance from the lungs and reduced survival. A bone marrow chimera experiment revealed that it is the CRAMP from the bone marrow and not the structural cells that showed the antibacterial activity [172]. LL-37 is microbicidal against uropathogenic Klebsiella also [173]. RamA is a global transcriptional regulator in Klebsiella genome and is associated with the regulation of 68 genes. It is reported to be important for bacterial response to AMP. It directly binds to lipid biosynthesis genes leading to lipid modifications, thus reducing the susceptibility of the pathogen to LL-37, colistin, polymyxin etc. Adhesion of *K. pneumoniae* and its uptake in macrophages is reduced with increased levels of RamA, and this is confirmed also in the in vivo infection studies [174]. Like HBDs, the CPS of *K. pneumoniae*also binds to LL-37 and escapes its antimicrobial activity [175]. CPS is responsible for the pathogen resistance to AMPs like LL-37 and HBD-3 in the lungs of cystic fibrosis patients [176].Use of colistin as a therapy for *K. pneumoniae* may confer cross-resistance to host AMPs like LL-37 [177]. Some of the synthetic AMPS effective against *K. pneumoniae* are enlisted in table. However recently two synthetic AMPs from tilapia piscidin 3 (TP3) and tilapia piscidin 4 (TP4), are found and their activity is checked against *K. pneumoniae* producing NDM-1 in a murine model. They are found to improve the survival and TP4 is found to be more effective than tigecycline. Both the peptides are found to non-toxic [178]. Again synthetic derivatives as we know from the literatures, hold a lot of promise in therapeutics of infection. Novispirin G-10 is a cathelicidin analogue which is tested for its toxicity in murine lung infection model of *K. pneumoniae.* The results show the peptide to be non-toxic in saline treated mice but highly toxic in Klebsiella infected mice. This shows the relevance of testing a synthetic AMP as a therapy in infection model [179]. Thrombin induced human platelet derived AMP has been used to produce synthetic AMPs PD1-PD4 and repeats of Arg-Trp (RW1-RW5) shows microbicidal activity against *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*. They can be used synergistically or as a peptide cocktail to further enhace the antimicrobial activity [180, 181]. Synthetic analogs of hymenochirin, AMP from the Congo dwarf clawed frog *Hymenochirusboettgeri*, named hymenochirin-1B-4B is found bactericidal against *E.coli, P. aeruginosa, K. pneumoniae, and S. aureus C. albicans*and has relatively less haemolytic activity [182]. A series of 36 synthetic AMPs has been screened for their activity against a group of pathogens like *P. aeruginosa, K. pneumoniae, A. baumannii,Enterobacter species and S. aureus*. All these AMPs contain unnatural amino acids.These peptides have MIC against these pathogens in the range from >100 to 6.25 μg/mL [183].

*Pseudomonas aeruginosa:P. aeruginosa*is a pathogen associated with airway colonization and is a major cause of morbidity and mortality as in cystic fibrosis (CF). Human β-defensins play an important role in the airways and is critical for innate defence against P. aeruginosa. However, the CF patients fail to display AMP activity against this pathogen [184]. Mucoid*P. aeruginosa*induces HBD-2 in the airway epithelial cells, underscoring its importance in the lung infections with this mucoid pathogen that may be important for CF patients [185].Similar observations are found when patients with chronic lower respiratory tract infection caused *by P. aeruginosa* are screened for HBDs in the epithelial lining fluid; HBD-1 and -3 are higher in these patients and HBD-2 and -4 are found in the detection range in contrast to lower than detection levels in controls [186]. HBD-2 also ameliorates lung injury caused by *P. aeruginosa* in the infected rat model [187].Similarly, murine synthetic mBD-3 is also able to kill the bacteria [188]. BCG cell wall proteins also helps in clearing *P. aeruginosa* in rat model by induction of HBD-1 [189]. There is a report that shows association of HBD-1 gene polymorphism with *P. aeruginosa* lung colonization in CF [190]. In CF patients, reduced expression and function of HBD-2 also plays a role in *P. aeruginosa* infection [191]. Diffuse parabronchitis (DPB) is a lung disorder associated with frequent superimposed infections of *P. aeruginosa*. HBD-1 and HBD-2 are found in high concentrations in the bronchiolar alveolar fluid (BAL) of DPB patients, and only HBD-2 is detected in elevated levels in the plasma. Synthetic HBD-2 has dose-dependent bactericidal activity against *P. aeruginosa.* However, treatment of these DPB patients with macrolides reduced BAL fluid concentrations of HBD-2 [192]. In other pulmonary diseases like pulmonary alveolar proteinosis (PAP), BALF levels of α-defensins and HBD-1, HBD-2 are found elevated. Thus in interstitial lung diseases, the kinetics of α- and β-defensins in lung lumen have significant role to play in bacterial infections [193]. In the keratinocytes, *P. aeruginosa*induces HBD-2 in an NF-KB dependant manner [194]. Like HBD-2, HBD-3 also has fast (20 mins) bactericidal activity against the MDR-resistant strains of *S. aureus*, *E. faecium, P. aeruginosa*, *S. maltophilia, and A. baumannii*, and the presence of human serum reduces the activity of HBD-3 against all these gram negative pathogens[194]. The flagellin of this opportunistic pathogen also triggers the innate immune system. The statically grown culture supernatants of *P. aeruginosa* are capable of inducing HBD-2 in the keratinocytes, indicating that a soluble factor may be responsible for this effect, which was found to be flagellin. The rhamnolipid in the outer membrane of the pathogen causes the flagellin to shed [195].This rhamnolipid present in the *P. aeruginosa*culture supernatants inhibits HBD-2 induction by the pathogen flagellin in the keratinocytes by inhibiting the calcium-signalling pathways and protein kinase C activation [196].HBD-3-carbohydrate-binding domain is very effective in killing *P. aeruginosa*PA14 strain and also represses its biofilm activity alone or in synergy with antibiotics [197]. P. *aeruginosa*also causes keratitis. In the cornel infection model, murine β-defensins -2 and -3 further promotes resistance to the pathogen [198]. Contact lens exposure, which is a major cause of *P. aeruginosa*keratitis inhibits HBD-2 induction in response to the pathogen. Murine β-defensin3 (mBD-3) is an ortholog of HBD-2 and mBD3 knockout mice succumbe more easily to infection and have reduced capacity to clear *P. aeruginosa*[199]. *P. aeruginosa*infection is common in burn patients. The immunosuppressive neutrophils of the burn patients are

incapable of inducing HBD-2 and -3 in response to proinflammtory stimulus, and are thus defective in the killing of *P. aeruginosa*[200].Lineage(-)CD34(+)CD31(+) cells from severely burned patients have inhibitory activities on HBD-1, and neutralisation of IL-10 and CCl-2 with the monoclonal antibodies have nullified the inhibitory effects [201].α- and θ-defensins can kill the ciprofloxacin resistant *P. aeruginosa* [202]. LL-37 alone or in combination with antibiotics also show anti-biofilm activity against this pathogen [203] It has been established in vitro that phenylbutyrate, a potent inducer of LL-37 and HBD-1 in the bronchial epithelial cells, when used with vitaminD3 inhibits P. *aeruginosa* [204]. LL-37 renders the antibacterial effects by enhancing cell stiffness and reducing transepithelial permeability that prevents epithelial invasion by P. *aeruginosa* [205]. The D-enantiomer of the peptide is also equally effective on the pathogen [206]. The airway surface liquid of CF patients is acidic and it is found that in acidic pH, LL-37 has attenuated killing capacity against *P. aeruginosa* [57]. LL-37 enhances virulence factor expression and antibiotic resistance to fluoroquinolone and aminoglycoside in *P. aeruginosa* PAO1 [207]. LL37 and certain synthetic derivatives of cationic steroid antibiotics (CSAs); CSA-13, CSA-90, CSA-131 are effective against hypervirulent strain of P. *aeruginosa* LESB58 with CSA-13, CSA-131 having anti-biofilm activity also. The LL-37 activity is impaired in presence of purified bacteriophage Pf1, which is highly expressed by the LES strain, but CSAs activity are unaltered with this virus [37]. 25-hydroxyvitamin D_3 loaded poly($_L$-lactide) (PLA) and poly(ε-caprolactone) (PCL) fibres are released on plasma treatment, which can induce higher rates of LL-37 in human keratinocytes and monocytes without any cytotoxic effects [208]. Some surfactants (bovine lipid-extract surfactant (B) supplemented with different AMPs like CRAMP, LL-37, CATH-1, CATH-2 and are tested for their AMP activity on MRSA and *P. aeruginosa*. However the CATH-2 involved sufactant mixture is found most effective as per in vitro assays [209]. Synthetic truncated fragments of LL-37 are made and LL-19, LL13-31, and LL7-25 are found to have anti-biofilm activity against P. *aeruginosa* PAO1 and most potent of them are LL7-37 and LL-31; LL7-31 have both strong antimicrobial and antibiofilm activities [210]. IG-25, a truncated version of LL-37 attached to a zido-capped poly(ethylene glycol) chain, is 18 fold more effective against *P. aeruginosa*compared to LL-37, but has no cytotoxicity [211].

Helicobacter pylori: H. pylori is the etiologic agent for peptic ulcer and chronic gastritis, and amongst the major risk factors for gastric cancer [212]. Scant literature is available about the effects of α-defensins on *H. pylori*. Elevated levels of HNP1-3, perhaps released from the infiltrating neutrophils are found in the gastric juice of *H. pylori*-infected individuals [213]. Similar observation was made with HBD-2 [214], the expression of which was restricted to the surface epithelial cells of both healthy and inflamed stomach as studied by Immunohistochemistry and in-situ hybridization. Higher levels of HNPs and HBD-2 are found in chronic gastritis and gastric ulcer compared to duodenal ulcer and healthy individuls [215]. HBD-2 expression showed an inverse relation to that of HBD-1, which correlated with the levels of bacterial colonization and inflammation [214, 216, 217]. While similar correlation is not found for HBD2, another report suggested that the degree of corpus and antral gastritis and HBD-2 upregulation was mutually connected and HBD-2 levels are restored after the eradication of *H. pylori* [218]. HBD-2 indeed inhibits the growth of *H. pyloriin vitro* and complete growth inhibition is achieved at 10^{-5} M [219, 220]. Investigators have reported an essential role of NOD1 in HBD-2-mediated killing of *H. pylori* in the gastric epithelial cells [221]. cag pathogenicity island of *H. pylori* encoding a type IV secretion system downregulates TNF-α-induced HBD-2 through inhibition of NF-κB in the human gastric epithelial cells [217].NF-κB p65-p65 homodimer binds to the HBD-2 promoter [220]. HBD-3 is also highly active against *H. pylori* and is readily induced during the early stages of infection. The induction is regulated by EGFR activation, which in turn activates MAP kinase and JAK/STAT pathways. However, HBD-3 is downregulated by persistent *H. pylori* infection due to the activation of tyrosine phosphatase, SHP-2 by CagA and may contribute to the chronicity of *H. pylori* infection [222].HBD-4 expression is low in healthy stomach, but increased in gastritis due to cagA-positive *H. pylori* infection or non-infectious aetiologies [223]. LL-37 is also induced in *H. pylori*infection [224][225] and cholesterol plays an important role in conferring antibiotic and LL-37 resistance to *H. pylori* [226]. A synthetic CSA, ceragenin CSA-13 possesses anti-*H. pylori* activity; while it is found to be resistant to gastric mucin, it loses activities when incubated at the low pH of the gastric juice as opposed to LL-37 that retains its antibacterial potential [227].

3.3 Antiviral role of AMPs

According to Wagner *et al*, viruses sustain in the evolution due to capability of genetic variation and varieties of transmission mode as well as efficient replication and persistence in the host [228]. These features make anti-viral therapy a difficult task. Despite the availability of several effective anti-viral drugs, the search for new anti-viral compounds is an active field of research to combat the emerging viral pathogens. As for bacterial pathogens, AMPs are suitable and potential alternative targets for the development of new therapies for viral infections. The promiscuity of AMPs allows for the validation of their broad spectrum anti-viral activities. In 1986, α-defensin is reported to exhibit anti-viral role against a number of viruses like HIV, HSV-1and -2 and CMV, and HNP-1 is found to be active against vesicular stomatitis virus in vitro[229]. Currently, there is a huge literature that refers to the anti-viral roles of α-, β-, θ-defensins and cathelicidins and synthetic peptides derived from them.

Human immunodeficiency virus (HIV): AMPs, especially defensins are very important in terms of anti-HIV activities. HNP-1,-2,-3 binds to gp120 and inhibit HIV-1 replicationin vitro. HNP-1 also inhibits viral entry mechanisms like binding to CD4 and refolding of env protein; subinhibitory doses of HNP-1 increases the half-life of

gp41 intermediates, thus potentiating the effects of neutralising antibodies against gp41 intermediates[86, 230][231]. However, HNPs are not anti-HIV factors for CD8 cell infection [232]. On the other hand, HD-5 and HD-6 increases the attachment of HIV viral particles to host cells and thus promotes viral infectivity. In contrast, others found interference with the binding between the envelope glycoprotein, gp120 and CD4 receptor by HD5. At high concentrations, HD5 downregulates CXCR4 coreceptor [233, 234]. θ-defensins also have anti-HIV activity and bind gp120 of HIV-1 to block the entry of R5 and X4 viruses [235].The most potent θ-defensin is retocyclin 2, which binds to gp120 with an affinity that is almost twice as that of HNPs [235].RC-101, a θdefensin derivative, prevents HIV-1 infection in human cervicovaginal tissues and retains full activity in the presence of vaginal fluid for nine days, making it a promising topical microbicide for HIV-1[41].However, different defensins have different mechanisms to stop HIV infection. Cyclic θ-defensinRTD-1 prevented HIV-1 entry, while HNP-1 and HBD-2 inhibited viral replication. All of them, however, decreased CXCR4 [236].Vaginal lavage fluids of women suffering from bacterial vaginosis are deficient in AMP activities as compared to the fluids from healthy controls or women with vulvovaginal candidiasis. This deficiency may predispose to HIV infection [237]. A study from our own laboratory showed no significant relations between the levels of AMPs (HNP-1 and HBD-1,-2,-3) in the CVL (cervicovaginal lavage) and HIV infection status of married Indian women whose husbands were HIV positive. Co-expression of the AMPs differs between the women living in HIV concordant and discordant relationships. There also exists an inverse relation between IL-8 and HBD-3 levels in the CVL of HIV-infected women [238].β-defensins in HIV-1 exposed, but uninfected individuals may contribute to the natural resistance mechanisms against HIV infection [239].LL-37 also has activities against HIV-1 replication in PBMCs and $CD4^+$ T cells [240]. The cathelicidin inducer Vitamin D is very closely associated with HIV-1 infection. A study based in Germany demonstrated that 47.6% of HIV patients have less than 20 ng/ml of 25(OH)D in the serum [241].Another study executed in the US on HIV^+ adults showed less than normal serum levels of 25(OH)D in 17% individuals, while 11% had very low levels of $1,25(OH)_2D$ in the serum [242]. Similar observations are made in a Norwegian study [243]. Currently, there exists no study, which clearly establishes the relationship or a link between vitamin D, cathelicidin and HIV. Different synthetic fragmented derivatives of LL-37 are screened for anti-HIV activity; FK-13 is the smallest one with potential to inhibit HIV and GI-20 is the one with the highest therapeutic index (twice that of LL-37) [244].Enfuvirtid made by Hoffmann-La Roche is an anti-HIV drug, which is retracted due to controversies regarding the cost of production and side effects [245].

Human papillomavirus (HPV): HPV is the most common infection among the sexually transmitted diseases (STDs). High risk HPVs are HPV 16 and 18, while the low risk ones include HPV 6 and 11. High risk HPVs can cause cervical cancer and the low risk viruses are associated with condylomata acuminata [246].The expression of HBD-2,-3 and LL-37 was described in the female genital tract in HPV-associated lesions [247].HBD-1 is not upregulated in anal intraepithelial neoplasia and anal condylomata acuminata caused by HPV in a group of homosexual men who were also suffering from HIV infection. However, HBD-2 and -3 are upregulated in this group, including HIV positive and negative men [248]. Another study found HBD-1 upregulation in HPV lesions of respiratory tract indicating that HBD-1 may have important role in HPV defence at specific anatomic sites [249]. HBD-1 gene polymorphism is associated with HPV susceptibility in Brazilian women population [250]. A study by Erhart *et al* showed that the expression of HBD-1, -2, -3 and psoriasin is significantly enhanced in HPV-infected vulvovaginal lesions, whereas LL-37 and RNase7 remain unaltered. Thus, it's tempting to conclude that these AMPs are involved in HPV infection in the female lower genital tract[251].A recent report suggests a role for HNP-1 in the therapies of HPV- induced condylomata acuminata [252]. Defensins can attract immature DCs which are deficient in the uterine cervical cancer. HNP-2 can reverse this deficiency by recuiting DCs in HPV-associated neoplatic lesions in vitro as well as transplanted in vivo [253]. HNP1-3 and HD-5 are potent antagonists of HPV infection. In contrast, HD-6 showed no anti-HPV activity. HD-5 is very effective against sexually transmitted HPV types. These α-defensins are present in the female genital tract at concentrations that exhibit anti-HPV activity in vitro and block virion escape from the endocytic vesicles.

4. Conclusion

In the face of global spread of multidrug resistant human pathogens, multitasking molecules like AMPs with both anti-infective and immunomodulatory functions are loaded with significant promise to be used as new therapeutic agents. However, even after two decades of thorough research, AMPs have not been able to fulfil the expectations to combat the 'difficult to treat' infections. To further complicate the situation, pathogens have evolved smarter ways by engaging different techniques to evade the host innate defence mechanisms. Detailed understanding of the mechanisms of resistance in microbes against AMPs helps to design new innovative therapeutic peptides. Based on this, synthetic mimics of natural AMPs have been developed, which may provide a solution to the existing problem. However, the progress made in this field remains slow and despite a large number of pre-clinical and clinical trials conducted with these AMPs, only a handful received FDA approval. Some of the AMP-based drugs available in the market are gramicidin S, daptomycin, nisin etc. Most of them are restricted to only topical applications. Omignan, pexignan and isegnan proceeded to phase III trial, but none of them managed to get the FDA approval. Major issues regarding the oral and systemic formulations of AMPs are high cost of production, bioavailability, pharmacokinetic stability, toxicity etc. Sequential and systematic execution of novel peptide design approaches and machine learning techniques to reduce the

high production cost, chemiinformatics tools, advanced technologies in peptide drug screening and high throughput peptide library screening, peptidomics, multifactorial toxicological studies, prodrug formulationetc will tilt the balance in favour of the AMPs in the battle against refractory pathogens.

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