ANTIMICROBIAL PEPTIDES IN THE TREATEMENT OF DERMATOSIS

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

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Ethics Statement

This study does not involve any kind of animal and human trial.

Abstract

Dermatosis refers to any skin defect or lesion on the skin due to scratches, wounds, ulcer, sunburn, skin abnormalities, skin thinning, different extrinsic and intrinsic factors and results allergy, skin infection, auto-immune disorder. For that skin protection decreases and it leads to damages of protective barrier and defensive system. Several conventional medication including antibiotics is using for dermatosis. But with uprising antibiotic resistance, the use of these antibiotics is compromised. The discovery or the design of antimicrobials with lower susceptibility to antimicrobial resistance is a key for the effective treatment of bacterial infections. Antimicrobial peptides appear to be promising therapeutic options for the treatment of dermatosis including soft skin tissue infections, atopic dermatitis and critical non-healing wound. Many peptides have broad spectrum antimicrobial activity against bacteria, fungi, and viruses. This comprehensive review highlights different skin diseases and conventional treatment complications, source of antimicrobial peptides and their potential activity against dermatosis causing microorganisms. Patients with diabetic mellitus are prone to have diabetic foot infection and critical non-healing wound. Some peptides work great to improve those condition. Also, Cathelicidin-DM can improve infected non-healing wound. Though, there are some limitations and challenges in AMPs development process for therapeutic purpose. But with more research on limitation, better understanding about AMPs, structural modification of AMPs can lead to develop a smart, safe and effective treatment.

Keywords: AMPs, Atopic dermatitis, Diabetic foot infection, soft skin tissue infection, LL-37, non-healing wound, cathelicidin-DM

Dedication

Dedicated to my respected and beloved parents and my supervisor Kazi Fatema Rahman for their support and guideline.

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List of Acronyms

AMPs ..... Antimicrobial peptides

AD..... Atopic Dermatitis

SSTIs..... Soft Skin Tissue Infections

TSLP ..... Thyme Stromal Lymphopoietin

IL..... Interleukin

DM ..... Diabetic Mellitus

DFIs .....Diabetic Foot Infections

T1D ..... Diabetes type 1

T2D ..... Diabetes type 2

Chapter 1

Introduction

Dermatosis refers to any skin defect or lesion on the skin. It is a disease of integumentary system and involves any area of skin and may involve the hair and nails as well. Skin having almost 1×8 m^2 surface area is the primary protective barrier between external environment and body. It's stratified keratinized epithelium provides a strong protection against infectious pathogens and allows body system to fight against the infection by secreting molecules against the exogenous pathogens (Rima et al., 2021). Skins defense decreases due to scratches, wounds, ulcer, sunburn, skin abnormalities, skin thinning, different extrinsic and intrinsic factors and results allergy, skin infection, auto immune disorder (Lee & Kim, 2022).

Common dermatosis including atopic dermatitis is caused by immune dysregulation, dysbiosis of skin flora, pathogen colonization, bacterial infection (Wang et al., 2021). It is a chronic inflammation skin disease which associated with the cutaneous hyperactivity (Lee & Kim, 2022). Atopic dermatitis includes Psoriasis caused by *Psoriasin, Erysipelas* (soft tissue bacterial infection), impetigo caused by *Staphylococcus aureus*, serratia marcescens infection, leprosy caused *Mycobacterium leprae*, herpes zoster, tinea pedis, chromoblastomycosis. Moreover cellulitis, folliculitia, pyoderma, soft skin tissue infections (Hu, 2019). Others common skin conditions like acne, epidermolysis bullosa, ringworm, vitiligo, diabetic foot infection, cellulitis (Pfalzgraff et al., 2018). Autoimmune disease like pemphigus, psoriasis, lupus are the conditions where healthy cells begin attack mistakenly by immune system (Marcinkiewicz & Majewski, 2016).

Antibiotics are commonly used for atopic dermatitis, soft skin tissue infection like diabetic foot infection, cellulitis etc (Chen et al., 2021). Combined formulation like corticosteroids and gentamicin, mostly used medication like fusidic acid, mupirocin, antiseptic like triclosan, chlorhexidine, retinoids, dithranol. Moreover, SSTIs medication like oritavancin, delafloxacin, tedizolid. Doxycycline, trimethoprim sulfamethoxazole also uses for moderate purulent infection treatment (Yoav Golan et al., 2021). Skin and soft tissue infections are mainly caused by the pathogen like *Enterococcus faecium, Klebsiella pnueumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii*, and Enterobacter species. These belongs to most recalcitrant bacteria, causes resistant to almost all the common antibiotic (Rima et al., 2021). With uprising antibiotic resistance, the use of

these antibiotics is compromised (Ventola et al., 2015). Different drug-resistant bacteria such as penicillin resistant, methicillin resistant, sulfonamide resistant, macrolide resistant, vancomycin-resistant, multidrug-resistant are identified and provides a serious challenge in the treatment of severe respiratory, topical, urinary & surgical site infections, bloodstream (Tacconelli et al., 2018).

Therefore, the design of antimicrobial peptides with lower susceptibility to antimicrobial resistance is a key for the effective treatment of bacterial infections (Chen et al., 2021). Due to various advantages of AMPs, it might be the replacement of conventional antibiotics. In recent years, research focused on the development of synthetic antimicrobial peptides (AMPs) with lower toxicity and improved activity compared to their endogenous counterparts. AMPs appear to be promising therapeutic options for the treatment of atopic dermatitis, soft skin tissue infections and wound as they show a broad spectrum of antimicrobial activity, low resistance rate and displays pivotal immunomodulatory as well as wound healing promoting activities such as induction of cell migration and proliferation and angiogenesis (Erdem Büyükkiraz & Kesmen, 2022).

The AMPs produced in human skin include defensins, cathelicidins, dermcidin, and other short proteins first discovered for other biological activities such as neuropeptides and chemokines. Many other larger proteins with direct antimicrobial action also can be found in the skin such as lysozyme, elastase, complement, S100 proteins, and others ((Rima et al., 2021). Although the sequences of AMPs are variable, many peptides have broad spectrum antimicrobial activity against bacteria, fungi, and viruses. They are mostly cationic & 20-60 amino acids chain in length (Rima et al., 2021). The cationic features of AMPs allow them to bind to negatively charged bacterial membrane molecules such as lypopolysaccharude and lipoteichoic acid. Extensive studies of these evolutionally conserved molecules have shown that they not only kill microbes but also have multiple functions against the host cells themselves (Rima et al., 2021). There are some obstacles in AMPs development process. Denaturation, degradation of protein and peptides, hydrolysis factors during transportation system, manipulation of hydrophobicity, homeopathic activity reduction due to micro encapsulation alone alginate by ionic gelation (Roque-borda et al., 2021).

1.1. Aim and objectives

This paper provides a comprehensive overview of antimicrobial peptides used in the treatment of dermatosis. The aim of this review article is

- To elaborately discuss about antimicrobial peptides as a new treatment option over conventional antibiotics
- To highlight the source and classification of antimicrobial peptides and their potentialactivity with mechanism of action.
- To outline their limitations and challenges
- To highlight improvements and future research directions in this field which can eventually result in frequent uses of AMPs in clinical settings.

1.2. Methodology

This paper has been written by using recent published articles, journals research papers. All necessary data has been collected from PubMed, Google Scholar, Research Gate, Frontiers in pharmacology, Nature journal, MDPI, Science direct, DermNet NZ. Also collected from Tandfonline, Biologics, Infectious Diseases Society of America (IDSA), Elsevier. There are some terms and definitions has been taken from google. Mostly used last five years article and journal, but some information also collected from previous 10-15 years to enrich the review paper. Keywords used to search for relevant papers include: AMPs, Atopic dermatitis, Diabetic foot infection, soft skin tissue infection, LL-37, non-healing wound, cathelicidin-DM.

Chapter 2

Dermatosis

2.1. Anatomy of skin

Skin is the largest body organ consisting of epidermis the most outer layer, dermis layer and hypodermis layer. Epidermis consists of keratinocytes, melanocytes, Langerhans cell, lipid layer (Kolarsick et al., 2006). The protective epidermis secret specific molecules against pathogens which undergoes to “innate immune” response followed by i) pathogen recognition ii) molecule induction to activate the host cell that will eliminate the pathogens iii) molecule secrets to kill pathogen (Yamasaki & Gallo, 2008). Also, langerhans cells transport antigen to regional lymph node and presents to native T lymphocytes, thus resulting immune response (Bezie et al., n.d.). Secondly dermis layer 30-40 times thick then epidermis. It contains migrant leukocytes, Lymphocytes, mast cell, tissue macrophage which helps in cutaneous immune response (Salmon et al., n.d.). Dermis layer consists of reticular dermis and papillary dermis, where reticular layer contains glands, blood vessels, lymphatics, hair follicles, fat sales and nerves surrounding elastin and collision fibers. Meanwhile papillary dermis consists of fibroblast cell, collagen, fat cell, nerve fibers, blood vessels, phagocytes that fight bacteria. Lastly, subcutaneous tissue also called hypodermis which consist fat and connective tissue (Kolarsick et al., 2006). In figure 1 there is shown the structure of skin including different layer of it (Kolarsick et al., 2006).

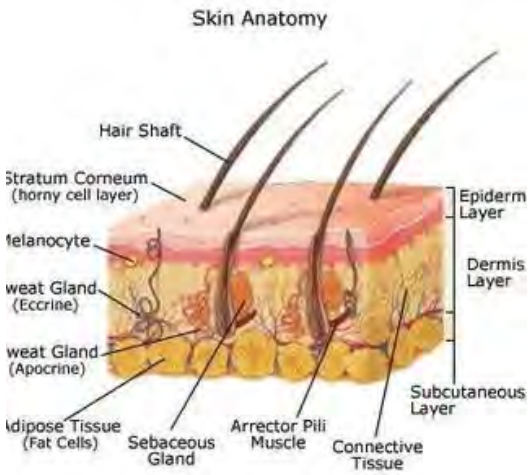

Figure 1: Skin anatomy. It includes epidermis, dermis and hypodermis layer with mentioning different glands and tissue (Kolarsick et al., 2006)

2.2. Dermatological infection and pathophysiology

Due to bacteria, fungi, contact eczema, scratch, wound, sunburn, abnormalities of skin, chemical factors, environmental factors, abnormal immune function, thinning pf skin, some chronic disease can lead to skin disease. Primary and secondary lesions can cause erosion, ulcer, atrophy, erythematous, crust, friable, excoriation (Bezie et al., n.d.). Mostly acute and chronic skin disease is caused by *S. Aureus, P.Aeruginosa*, group A-B *hemolytic streptococci*, Herpes simplex, *Trichophyton rubrum*, dermatophytic fungi. Soft skin tissue infections like impetigo, necrotizing facilities, cellulitis, folliculitis, Carbunculosis, abscesses, chronic wounds caused by *S. Aureus*. Diabetic foot infection is another soft tissue infection which associated with diabetes mellitus complications. Dermatophytes is superficial fungal infection is the most common condition. Atopic dermatitis is a chronic inflammatory skin disease associated with cutaneous hyperactivity due to environmental triggers. Chronic infection skin disease including psoriasis which is caused by Psoriasin (Herman & Herman, 2019) and Erysipelas caused by *S. Aureus* is mostly common. Impetigo which occurs mostly in children that also due to *S. Aureus*. Moreover, Serratia marcescens infection, Leprosy caused by *Mycobacterium leprae*, Chicken pox, Herpes zoster, Hand-foot-mouth disease, Tinea pedis, Chromoblastomycosis, mycoplasma infection are also bacterial skin infection (Wang et al., 2021b). Chronic skin disease may lead to systemic infection like respiratory tract infection urinary tract infection (Olesen et al., 2012).

2.2.1. Skin and soft tissue infection diseases

Skin and soft tissue infection is a bacterial infection of skin, connective tissue like tendons, ligaments. Bacteria are introduced when there is a small scrape, burns or cut that contaminated with soil or, saliva and leads to infection. Most common bacteria are S. aureus, streptococcus. Some common SSTIs are impetigo, cellulitis, necrotizing fasciitis, diabetic foot infection (Lipsky et al., 2017). In table 1 there are some SSTIs diseases with the name of pathogens, factor and symptoms have been shown (Lipsky et al., 2017).

Table 1: Skin and soft tissue infection diseases and it factors and symptoms (Templer & Brito,2009).

SSTIs diseases	Pathogens	Factors & symptoms
Impetigo	*Streptococcus pyogenes, S. aureus*	Occurs when in contact with sores, due to broken skin, warm, humid weather, other health conditions. Symptoms including reddish sore around nose and mouth, fever, rapid breathing.
Folliculitis	*S. aureus, Pseudomonas,* proteus	Due to follicle damage, abscesses in deeper subcutaneous tissue. Symptoms including pruritus, papules, pustule formation, fever, malaise
Diabetic foot infection	*S. aureus,* beta-hemolytic streptococci	Injured area becomes red, pain experienced. This infection can occur due to diabetes mellitus complexations.
Necrotizing infections	*S. aureus, S. pyogenes*	Patients with diabetes, chronic liver disease, other like varicella infections, penetrating injury, burns, due to muscles strain etc. Symptoms including swelling, pain, blisters, may develop anesthesia if superficial nerve is infracted.

2.2.2. Pathophysiology of Diabetic foot infection

Diabetic foot infection is a soft tissue infection which associated with diabetes mellitus complications. This infection generally caused by gram positive bacteria especially *S. Aureus*, beta hemolytic streptococci. The infection can be classified generally mild to moderate and sever condition. Among of all SSTIs, food infection is most common and serious complication associated with diabetes mellitus (Lipsky et al., 2017).

Mostly 30-50% patience with diabetes is susceptible to foot infection due to vascular insufficiency, decreased neutrophil function. Osteomyelitis is a serious and common complication patient with diabetic foot infections (Lipsky et al., 2017). If purulent discharge is present in ulcer or erythema or, tenderness in duration, warmth, pain confirm the diagnosis

of Diabetic foot infection. Also, other symptoms like failure in wound healing, foul order or the presence of necrosis can confirms the condition of foot infection (Lipsky et al., 2017).

Non-infected ulcer does not need antibiotics but for mild condition oral antibiotics like dicloxacillin, clindamycin, cephalexin is administered. Major condition is treated with intravenously by using clindamycin, ciproflixacin, tazobactam or imipenem. Consideration requires in the risk of methicillin resistant *Staphylococcus aureus* infection (Lipsky et al., 2017). For an infected foot of diabetic patient more or less tissue damage will be shown continuously like as figure 2.

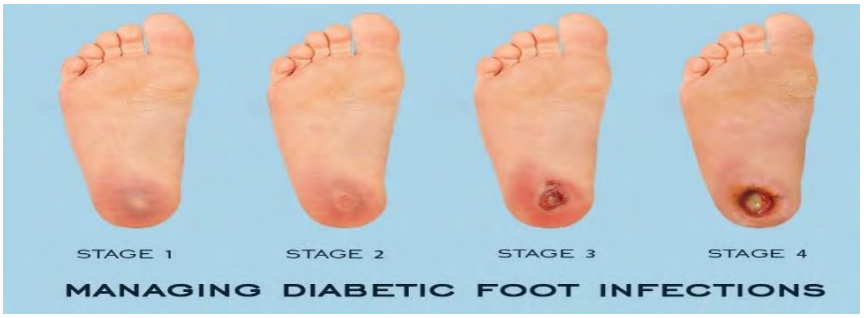

Figure 2: Diabetes foot infection**.** In stage 1 there is initial stage has shown, stage 2 is indicating high risk of having infection with damage skin. In stage 3 it's a result of ulcerated foot and stage 4 indicating infected with necrotic foot (Lipsky et al., 2017).

2.2.3. Wound management in Diabetic foot infections

Damage of skin protective barrier allows the pathogen for contaminate and forms colonization that eventually infect the sub-epidermal tissue (Bader et al., 2008). The colonized pathogen surrounded in the wound by the direct inoculation. The infection occurs in colonized wound directly connected with contaminated organism's inoculum size and its congenital virulence and oppositely related to host's defense ability. *S. Aureus & Staphylococcu*s both of species inherently drastic in wounds and causes damage to the skin. Wound is susceptible to infection when there is chances of local ischemia or foreign body or necrotic tissue increases (Bader et al., 2008).

Figure 3: The evaluation of superficial wound infections. The continuous growth of pathogens and colonization formation which is leading to initial infection (Bader et al., 2008).

Critical colonization is the intermediate stage between the colonization and the infection which is shown at figure 3. And severe infection leads to neuropathy predisposes. Almost all diabetic patients more likely to have complications of soft skin tissue infections (Bader et al., 2008).

Table 2: Antibiotic regimens in diabetic foot infection treatment (Bader et al., 2008)

Condition	**Medication and dose**	**Treatment duration**
Mild	I)Cephalexin 500mg/day;4times II)Dicloxacillin, 500mg/day;4times iii)Clindamycin, 300-450mg; 3 times iv)Sulfamethoxazole, 160/800mg;twice/day	Treatment duration 1to 2weeks, dicloxacilllin is choice for methicillin-susceptible S. aureus (MSSA). Cephelaxin is for penicillin allergic patients. Clindamycin also does cross resistant and erythromycin resistant, also does inducible resistance to methicillin-resistant S. aureus (MSRA).
Moderate	Nafcillin (1-2g)IV route , Cefazolin (1-2g)IV route, Vancomycin,30 mg/kg/day , Levofloxacin, Moxifloxacin, Ciprofloxacin, Ertapenem.	Depending on response treatment duration 2 to 4 weeks. Cefazolin is the choice for Penicillin allergic patients, Nafcillin is choice for MSSA, and vancomycin for MRSA.

Severe	Ciprofloxacin 400mg, IV route. Piperacillin, 3.375-4.500g, IV route Vancomycin 30mg/kg IV route, Ciprofloxacin 400mg, IV route.	Vancomycin choice for MRSA. Treatment duration 2 to 4 weeks.

2.2.4. Pathophysiology of atopic dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory skin disease which is caused by several environmental and genetic factors. This inflammation mainly damages the stratum corneum. Though the exact mechanism is unclear now, but due to complexity, barrier dysfunction, itch, inflammation can develop AD (Pfalzgraff et al., 2018). It is characterized by relapsing eczema with dry and itchy skin. In damage epidermis, keratinocytes secrets thyme stromal lymphopoietin (TSLP), interleukin (IL)-25, IL-33, these will produce type 2 immune deviation through OX4OL / OX40 signals (Pfalzgraff et al., 2018). Next the inflammation down regulates the development of (FLG) filaggrin in the keratinocytes & promote the dysfunction of epidermal barrier. Moreover, crosstalk occurs in keratinocytes and non-histaminergic sensory nerves, immune system is responsible for emerging chronic itch of AD (Pfalzgraff et al., 2018).

AD development focuses three steps including dysfunction of barrier, inflammation and itchiness. Due to barrier dysfunction, the total amount of ceramide level ratio of cholesterol, molecule like loricrin, filaggrin, involucrin level also decreases (Pfalzgraff et al., 2018). Thus, free fatty acid, esterified also shortened. In acute AD, abnormal number of IL-4, IL-13 have been shown, thus abnormal production of dendritic cell and more activation of dermal cell leads to more chemo-attractive agents like CCL17, CCL18, CCL12 (Nakahara et al., 2021). These agents are chemo-attractive to the Th2 cell generates IL-22 that is linked to inflammation and it is a potent inducer of keratinocyte proliferation. FLG & LORs well regulation and expression is important for effective skin barrier formation. There expression prevents by IL-4 & IL-13 and this is susceptible to gene conferring (Pfalzgraff et al., 2018).

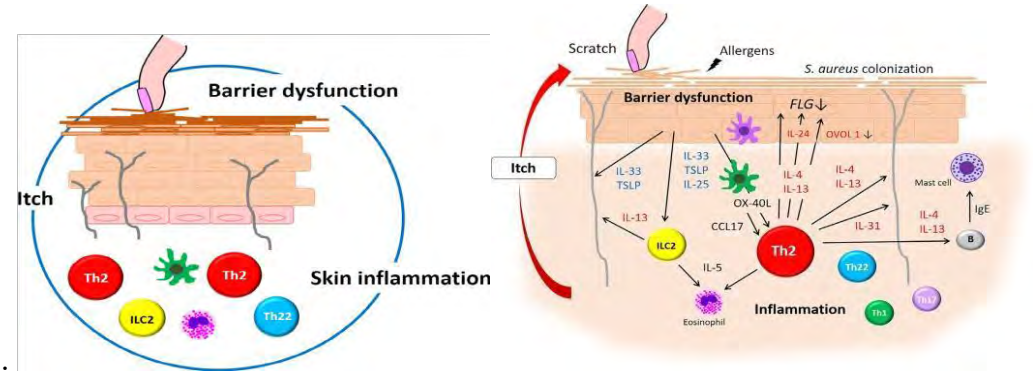

Figure 4: Barrier dysfunction, inflammation, and itch in atopic dermatitis. The total amount of ceramide level ratio of cholesterol, molecule like LOR, FLG, involucrin level also decreases. Abnormalities leads to chemo attractive agent production (Pfalzgraff et al., 2018).

2.2.5. Conventional treatment of dermatosis

Diabetic foot infection is soft tissue infection which associated with diabetes mellitus complications. Oral antibiotics including dicloxacillin, cephalexin, clindamycin, vancomycin, nafcillin, piperacillin, ciprofloxacin etc medications are used for diabetic foot infections (Bader et al., 2008). There are many antibiotic therapy for infected wound including aminoglycosides (neomycin, gentamicin), sulfonamides, metronidazole, as well as fusidic acid, retapamulin, mupirocin, hyperbaric oxygen are also used (Kaiser et al., 2021). Superficial fungal infection like dermatophytes are the most common condition which can be treated by ketoconazole, itraconazole. Atopic dermatitis treats with erythromycin, cloxacillin, corticosteroids, ketoconazole, salicylic acid, antibiotics (Bezie et al., n.d.). Also, AD is mediated by some cytokines like IL-4, IL-13, IL-17, thymic stromal lymphopoietin, calcineurin inhibitor as first-line therapy

2.2.6. Current unmet clinical need

Atopic dermatitis treated with cytokines like IL-4, IL-13, IL-17, thymic stromal lymphopoietin, calcineurin inhibitor as first-line therapy. Some potential side effects have been reported of these therapies such as impaired skin barrier functions, burning, atrophy, pruritus and serious infections. Also, hyperbaric oxygen has not been shown that much effective in wound healing process. Rather it can lead to side effect like myopia and oxygen toxicity in brain which can cause seizure pneumothorax. Antiseptic and topical antibiotics

need to reach with adequate concentration at the site of infected wound to kill or remove infected microorganisms. But due to poor penetration into the biofilms most medication cannot show effective results (Kaiser et al., 2021). There is no orally administered drug that can successfully cure MRSA, and also those infection associated with gram negative infections. Moreover, there is a so called "ESKAPE" which is an acronym of 6 bacterial organisms associated to multiple drug resistant. These includes: *Enterococcus faecium, S.aureus, K.pneumoniac, A.baumannii, P.aeruginosa & Enterobacter SPP.* . These multidrug resistant bacteria shows resistant to several antimicrobial drug or almost all approved antimicrobial drug agents (León-Buitimea et al., 2020). Furthermore, others including penicillin resistant, methicillin resistant, macrolide resistant, sulphonamide resistant, vancomycin resistant also has been reported in recent few years. These resistant leads to serious challenges in the treatment of severe respiratory, topical, urinary & surgical site of infections, blood infection (Tacconelli et.al.2018). Also, SSTIs condition like necrotizing infection and ischemia prevent delivery / or, therapeutic effect of antibiotic to the affected area. When a patient with SSTIs admits to hospital, they are at high risk of multidrug resistant organisms. It's a concern to antibiotic resistant of SSTIs patients causes by MRSA and also multidrug resistant common in CA-MRSA & HA-MRSA infection. Where HR-MRSA susceptible to the vancomycin, trimethoprim-sulfamethoxazole, linezolid. On the other hands CA-MRSA is susceptible to trimethoprim-sulfamethoxazole and a wide range of antimicrobial agent including clindamycin, tetracycline, quinolones oral drug, Streptococci also susceptible to penicillin. This microbs is now universally resistant to all drug. Nafcillin, dicloxacillin causes cell lysis and produce toxin (Templer & Brito, 2009). In addition, WHO has already been launched a least where they mention many bacteria that are almost resistant to various drugs. Bacterial resistance becomes Global issue as the line of resistance exceed to the inactivation of first line drugs. Thus, continuously leading high mortality rates. And this problem grows when defensive molecules generates by bacteria or they gain ability to resistance plasmid of others bacterium. That's why importance of new drug production using antimicrobial peptides have been raised highly (Thapa et al., 2021).

Chapter 3

Antimicrobial peptides

3.1. What are AMPs

Antimicrobial peptides refer as host defense peptides relates with innate immune response that found among mostly in all classes of life. They are very small peptides, 10-60 number ofamino acid residue, cationic in nature must but some anionic AMPs exists also (Atefyekta et al., 2021). They contain several acidic nature amino acids including glutamic acid & asparticacid with emerge of antibiotic resistant microorganism issue. AMPs has been good applications prospect in medication, animal husbandary, food, agriculture etc (Atefyekta et al.,2021). They are considered as new classes therapeutic agents that can provides therapeutic effect over conventional treatment and can tackle lots of challenges over invasive pathogen. Their unique feature with small molecular size, low resistance development, fast activity, lowtoxicity (Zupin et al., 2022). As, they are the part of innate immune system those properties are very similar in other kingdoms including insects & plants, so researcher showing interest and highly expecting new generation of drug over traditional / conventional treatment (Thapaet al., 2021).

3.1.1. Classification of antimicrobial peptides

AMPs can be classified based on

(1) Source
(2) Activity
(3) Structural characteristics, and
(4) Amino acid-rich species (Huan et al., 2020)

Classification based on sources

A) Mammalian Antimicrobial Peptides: The sources of AMPs can be found into mammals (human host defense peptides), microorganisms, insects, amphibians. Mammalian AMPs can be found mainly in human, cattle, sheep & in other vertebrates. The main families of them including Cathelicidin & defensins. Whereas Defensins are divided into α- defensins, β-defensins & θ-defensins based on disulfied bonds position (Reddy et al., 2004). Human host defense peptides (HDPs) provide protection to human from infectious pathogens and in human

growth they show different several expressions. Like example, cathelicidin-LL-37, is a famous & familiar AMP produce from human body, usually that can be identified in new borninfants skin. Then, human beta-defensins 2 is found in the elder age rather than young person(Sibel Akalın et al., 2014). Also, HDPs can be found in several parts of the body including ears, eyes, respiratory tract, mouth, lung, urethra, intestine etc. Moreover, AMPs found in human breast milk play an key role in the breastfeeding as it can reduce the morbidity & mortality of the breast feeding infant. Dairy is a key source of AMPs, are produced via milk enzymatic hydrolysis. Several AMPs are characterized from α-lactalbumin, lactoferrin, β- lactoglobulin, & casein fractions where most famous peptide is lactoferricin-B (Sibel Akalın et al., 2014). In addition to, AMPs derived from the dairy products can utilize for dairy preservation. Furthermore, antimicrobials function HDPs, like Cathelicidins & defensins affect the regulation of immune, wound healing, Apoptosis (Wang et al., 2014).

B) Amphibian derived Antimicrobial Peptides

Antimicrobial peptides from amphibians have an important role in providing protection to amphibian from foreign antigen & pathogens those Induces the decreased population of global amphibian population decline. Frogs, those are general source of amphibian AMPs & magainin is the most famous AMP among all of amphibian derived AMPs. the skin secretions of frogs from genera Silurana, Xenopus, Hymenochirus, & Pseudhymenochirus under the family of Pipidae contains high amounts of AMPs (Conlon and Mechkarska, 2014).

C)Insect-Derived Antimicrobial Peptides

Antimicrobial peptides mostly synthesized in bodies fat & insects blood cell. Strong Survivable capability & strong adapting system of insect are due to this main reason (Vilcinskas et al., 2013). Cecropin AMPs from insects is most famous and can be found mainly in guppy silkworm, Drosophila, bees, rosophila. This AMPs can shows activity against individual inflammation and cancers. Invasive harlequin ladybird and black soldier fly contains mostly 50 AMPs, whereas pea aphid can provide Lack AMPs, a peptide derived from bee royal jelly is called jellein, it shows great effects against several bacteria, fungi and lauric acid conjugated properties can prevent the parasite (Huan et al., 2020).

D)Microorganisms-Derived Antimicrobial Peptides

Antimicrobial peptides from microorganisms including bacteria, fungi and some familiar peptides including nisin, gramicidin which found in Lactococcus lactis, Bacillus brevis, Bacillus subtilis. As chemical synthetic AMPs has high price, the natural biological expression

gain attraction for uses. Specific species like *Pichia pastoris*, *Saccharomyces cerevisiae* and bacteria including *E.coli*, *B. subtilis* and plants are using in expression systems but due to toxicity, difficult purification, proteolytic degradation, production of AMPs is difficult in *E. Coli*. That's why it is necessary to take full advantage of using fusion tags (Yu et al., 2015). Also, several AMPs have been synthesized, extracted and storage from the seed, stems, seeds, plants leaves. They are also classified in several groups including snakins, thionins, defensins (Tang et al., 2018). Moreover, marine derived AMPs also shown to reported to the increasing value fixed via people to marine sources. Although, many of these AMPs during in vitro test shows hopeful results, and in in-vivo, like As-CATH4 have been shown effect of an immunity-stimulating & can increase the anti-infective ability in combination of AMPs drugs (Huan et al., 2020). In-vivo, like As-CATH4 have been shown effect of an immunity-stimulating and can increase the anti-infective ability in combination of AMPs drugs.

Classification Based on Activity

A) Antibacterial peptides

The contains a great capability against several pathogens including acinetobacter, MRSP in clinical drug, VRE, *S.aureus*, *E.coli*, Salmonella , vibrio-parahaemolyticus of aqualic product. Natural & synthetic AMPs including cecropin, nisin, defensins results good activity in prevention against gram negative bacteria & gram positive bacteria, inhibit MRSA with showing low toxicity (Huan et al., 2020).

B) Anti-fungal peptides

Many AFPs shows great anti-fungal activities over some common invasive fungi including *Candida albicans*, aspergillus, yeast and over filamentous fungus against mold of food & agriculture (Liscano et al., 2020). AurH1 which synthesized from aurein shows effective improvement in *C.albican* infections treatment. They also can prevent the growth of A.flavus that is harmful to human. Lactobacillus plantarum can produce 37 antifungal peptides. Moreover, chemically synthesized species shows inhibitory effect against *Z.bailii* and *Z.rouxii* (Liscano et al., 2020).

C) Antiviral peptides (AVPs)

HIV still now a long-term life threatening to human, but AMPs shows strong activity against virus. They show mechanism by-

1) Preventing attachment with host cell receptor to virus.

2) Destroys viral envelope or by,

3) Preventing virus replications. (Jung et at., 2019).

A recently reporting AMP Epi-1 that has been shown great inhibitory effect over foot and mouth disease virus. Most important anti HIV peptides including alpha and beta defensins, LL-37, caerin 1, gramicidin D, maximin 3, dermaseptin-S1, magainin 2 siamycin-1, siamycin-ii, fuzeon etc. COVID-19 causing virus requires viral spike protein for infectivity. Some fusion inhibiting peptides associated to S protein to interfere in its folding, thus prevent infections (Huan et al., 2020).

D) Anticancer peptides

Magainin II, BR2, Buforin II b are the tested anticancer peptides. There are mainly two types anticancer peptides including active against bacteria, mammalian & cancer cell (Huan et al., 2020). And another one assemble to those active over bacterial & cancer cells. These are referred as dual antibacterial-anticancer activity showing peptides. Anticancer peptides are positive net chargeable, high hydrophobicity containing (Huan et al., 2020).

E) Anti-inflammatory peptides

FGL peptide has the ability to modulate hippocampus inflammation. Another one, Lactoferricin which can be found from proteolytic cleavage from lactoferrin via pepsin at acidic conditions and shows anti-inflammatory properties (Huan et al., 2020).

F) Antidiabetic peptides

These peptides exerts their activity by modulating G-protein coupled receptor kinase, or by activating GLP-1(glucagon) receptor. Though it is not sure till now, research is going on this concept (Huan et al., 2020).

Classified based on Amino Acid-Rich Species

A) Proline-Rich peptides. Proline acts by entering to bacterial cytoplasm using transporter SbmA without killing bacteria via membrane disruption. After that proline blocks aminoacyl-to TRNA binding to peptidyl-transferase center by targeting bacterial ribosome (Huan et al., 2020).

B) Tryptophan, Arginine Rich AMPs Tur1A-AMPs from tursiops truncates disrupts the transition of protein synthesis via binds with ribosome. Moreover, proline-rich AMP from crab shows activity against gram positive, negative bacteria (Huan et al., 2020).

C) Histidine-Rich peptides

They are goon in membrane permeation activity .For example, HV-2, L4H4, HV2 peptides induces the bacterial cell membrane permeability thus causing cell membrane rupture & death in result. They also prevent the production of TNF-X (Tumor necrosis factor) and results the inflammation stops. L4H4 is a great anti-bacterial molecule and they can penetrate the bacterial call via 4 histidine insertion (Huan et al., 2020).

D) Glycine-Rich AMPs

R-group of glycine is non-polar amino acid atlacins, diptericin AMPs contains 14-22% glycine residue (Huan et al., 2020).

Classification based on Structures

They are 4 categories including

a) Alpha-helical
b) Beta-sheet
c) Linear extension structure
d) Both alpha & beta sheet peptides (Huan et al., 2020).

A) Alpha-helical peptides

These are found from mammalian, insect, plant, animal, fish, amphibians. This structure depends strongly to membrane contact. Acquiring membrane target activity, they need to adopt of amphipathic configuration (Huan et al., 2020). Their structure make them able to create pores in membrane and allows agents to insert. Its hydrophobic region connect with membrane lipid-core through membrane bundles. Whereas, hydrophobic portion attach within inner membrane area via pore formation. This group includes magainin, moricin, melitlin, cecropin etc (Huan et al., 2020).

B) Beta-Sheet AMPs

This structure allows them to forms two strands and the structure depends on disulphide intramolecular bonds. Defensin is a beta-sheet AMPs which has disulphide bond that give

stability to the structure and prevent destruction of protease medication. Whereas, it's hydrophobic part is cationic and generally associated with the antibacterial activity (Q.Y. Zhang et al., 2021).

C) Linear extension structure

These peptides contain high tryptopham content, glycine content and high content of proline. For example: Indolicin (Q. Y. Zhang et al., 2021).

D) Both alpha and beta sheet peptides

This group having alpha helices & beta sheets are highly affinity to membrane considerable to other groups. For example: Defensins that interfere with the sphingolipids of fungal membranes are the most familiar, exhibiting the antifungal activity. Neurospora crassa have antifungal peptide which is pisum sativum defensin-1 that can interact with fungal and yeast glucosylceramides, thus causes death of cells in *C.albican* via activating signaling cascade associated with reactive O2 species (Huan et al., 2020).

3.2. Mode of action

AMPs exerts their mechanism providing two types by -

i) Membrane targeting

ii) Non-membrane targeting mechanism (Huan et al., 2020).

i) Membrane targeting mechanism

This activity can be described via different model including Toroidal model, Barrel-Stave model, Carpet like model which also shown in figure 5.

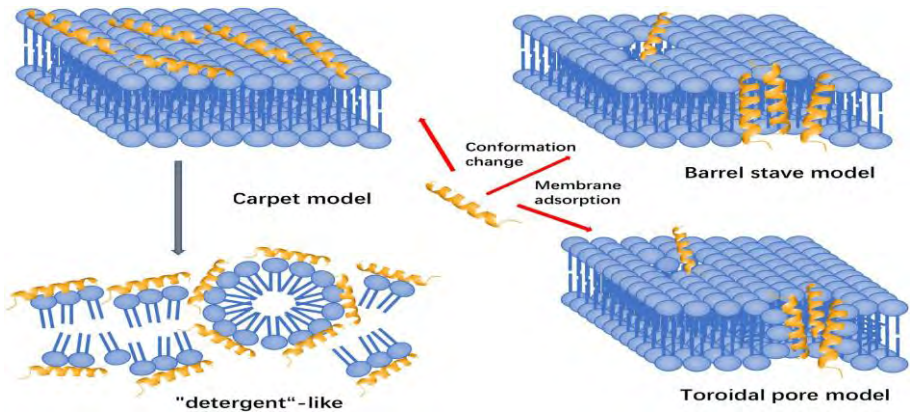

Figure 5: Action of extracellular AMPs activations. AMP is stored on the surface area and destroying cell membrane acting like detergent. They are arranging in parallel way when inserted in the bilayer of cell membrane and creates a channel to make a hole in membrane (Huan et al., 2020).

The Toroidal model

Known as wormhole model also. AMPs vertically attached in cell membrane and crew or, bend to ring hole formation around 1-2 nm diameter. For example: Lacticin Q, magainen 2, arenicin etc. Moreover, cationic peptides including TC19, TC84, BP2 also compromise membrane barrier via fluid domain creating (omardien et. al., 2018).

Barrel-stave model

AMPs assemblage with each-other, insert into cell membranes bilayer forming multimers that forms a channel and results in cytoplasmic outflow. Most of the severe cases, AMPs causes cell death via collapsing cell membrane. For example: Alamethicin functions pore forming process via this model (Lipkin, 2015).

Carpet like model

They arranged parallel structure around the cell membrane. The hydrophilic portion comes near the solution part & hydrophobic portion comes near the phospholipid bilayer. Then surrounds the membrane surface and destroy cell membrane. This is detergent like actions. For example: LL-37 (Huan et al., 2020).

Anti-biofilm peptides exert their mechanism by

i) Signal degradation within biofilm.
ii) Permeable capability in cytoplasmic membrane
iii) EPS production modulating (Huan et al., 2020).

Non-membrane targeting mechanism

AMPs directly penetrate the pathogens cell and reach to cytoplasm, they can show several inhibition mechanisms (Huan et al., 2020).

A) Inhibition of protein biosynthesis

After targeting the cell membrane AMPs interfere with transcription and translation related enzyme and factor. For instance, Bac7 1-35 targets to ribosome for interfering translation. Another one, Tur-1A interfere with protein synthesis by preventing transition from initial to extension phase. Different AMP affects protein biosynthesis via interacting in intracellular pathway (Huan et al., 2020).

B) Inhibition of Nucleic acid Biosynthesis

In this mechanism, AMPs induce the degradation of nucleic acid thus results disrupt biosynthesis of nucleic acid. For example: Indolicidin (Huan et al., 2020).

C) Inhibition of Protease Activity

By preventing protease activity, AMPs can inhibit metabolic activities of pathogens. For example, histatin 5, Indolicidin, NAP-2, Cathelicidin etc. Cathelicidin-BF effectivity prevent thrombin-induced platelet aggregation thus results in prevention of protease activity (Huan et al., 2020).

D) Inhibition of Cell Division

AMPs prevent DNA replication which blocks cell cycle and causes block to chromosome separation. For example, AMP having 20 amino acid can show affinity by interfere with pathogen's DNA replication via producing dysfunction to S-Phase in cell cycle. That's how it exert effect specially to kill *C.albican.* Moreover, AMPs damage organelles of fungal cell. For example, Histintin-5. Other AMPs targets (LPS) lipopolysaccharide of pathogen's cell wall. This LPS contains negative charged phosphate group which makes a salt bridge. The salt bridge makes an electrostatic network by using Ca2+, Mg2+. This network provides barrier against to hydrophobic antibiotic, thus causes low permeability to cell wall, AMPs target those LPS and reduce the pathogens protection barrier (Huan et al., 2020).

3.3. AMP applications in the Critical Priority Bacteria

i) *A.baumannii* generates resistance by non-enzymetic or enzymetic route. It can cause pneumonia & dermal, urethral infection. But this bacteria contains reservoir of resistance gene that is very difficult to be controlled recent different studies shows that these bacteria can be eliminated by using AMPs (Roque-borda et al., 2021).

ii) *P.aeruginos* which causes lung inflammation by releasing IL6 & TNF-alpha with the help of LPS. This also associated with the cystic fibrosis & chronic obstructive pulmonary disease. These bacteria use biofilm forming and causes resistant to most of the medication with antibiofilm action containing AMPs can shows potential effect on these bacteria (Roque-borda et al., 2021).

iii) Due to case of resistance, Enterobacteriaceae can induce carbapenem resistant. *K.pneumoniae* multi drug resistant shows by high complex bacterium and making difficulty to treat. Thus, increasing fatality rate in human. Polymyxin AMPs approved by FDA can improve the associated problems (Roque-borda et al., 2021).

iv) *M. Tuberculosis* is causing higher fatality rate in human recently with its advanced capsule composition containing peptidoglycan. It can remain in body without converting for many years. As a result many conventional drug is not effective and showing adverse effect. Human beta-defensin 2&3, cathelicidin LL-37 can fight against this Tuberculosis (Roque-borda et al., 2021).

v) Recent study showing that brevinin-1 AMPs can selectively kill only intracellular bacteria and without causing any damage to the macrophage & overall hydrophobicity is showing 65% (Roque-borda et al., 2021).

3.4. AMP against High-Priority Bacteria

This group includes the bacteria those are vancomycin-resistant, methicillin-resistant, clarithromycin-resistant, fluoroquinolone-resistant, third generation cephalosporin-resistant.

i) During study, it was found that Anoplin AMP and its analogue were modified by changing amino acids different position. These give an excellent outcome to better interact with bacterial lipid membrane (Roque-borda et al., 2021). ii) BF2 peptide using against 2ATCC strain shows antimicrobial activity against the strains and also shows a fractional inhibitory concentration for ATCC strain with teicoplanin combination (Roque-borda et al., 2021). AMPs are the small molecular with multi-functional activity including several therapeutic functions like anti-inflammatory, endotoxin-neutralizing activity, immunomodulatory, cytotoxic effect in cancer cell (Erdem Büyükkiraz & Kesmen, 2022). They mainly function via interacting cell membrane of microbial with receptor-mediated interaction, or the non-receptor mediated interaction (Erdem Büyükkiraz & Kesmen, 2022).

Chapter 4

Therapeutic potential of AMPs

4.1. Diabetic foot infection

Patients associated with diabetes are easily affected by food infections. These infections can be classified as mild moderate and saver condition. Generally, *S. Aureus*, Beta hemolytic, streptococci cause this type of condition (Depta et al., 2022). Most diabetic foot infections begin with ulcer condition, or localized cellulitis, and necrotizing facilities can be developed in absence of the ulcer or, traumatic injuries. Patients having diabetes are susceptible to this infection due to neuropathy, neutrophil dysfunction, direct related with impaired body defense mechanism. As a result, the wound is spread superficial to subcutaneous and even deeper (Bader et al., 2008). Presence of purulent discharge from ulcer, pain, warmth, induration, tenderness, foul odor, presence of necrosis, failure of healing any wound are the indication of diabetic foot infection (Depta et al., 2022).

Diabetes mellitus patient suffers with hyperglycemia because of impairment and defective insulin secretion. These patients also have microvascular complication that affects ultimately eyes, nerves, kidneys and also cardiovascular diseases increases and due to long term hyperglycemia, it leads to blood vessels damage and decrease vessels elasticity, overgrowth, increase atherosclerosis. And this ultimately leads to diabetic foot syndrome (Depta et

2022).

I) Role of AMPs in Diabetes Mellitus type 1

This is the DM type 1 chronic & auto immune disease due to damage of beta-cell that produce insulin due to insufficient insulin disrupts GIT functions and leads to T1D complication including intestinal enteropathy, gastroparesis, non-alcoholic steatohepatitis (Depta et al., 2022). T1D patients also lacks of AMP compared to healthy people because beta-cell producing insulin also responsible for cathelicidin related AMPs production. A study from 2015 found out that, AMPs shows positive effects on functioning and regenerating pancreatic islets. Cathelicidin AMPs also shows regulations of intra island communication by acting as autocrine & as a paracrine factor stimulating in secretion of both insulin and glucagon (Depta et al., 2022).

II) Role of AMPs in Diabetes Mellitus type 2

DM type 2 caused by elevated glucose levels in blood with insulin resistance, dysfunction of beta-defensin, increase glucose secretion by liver. AMP activated protein kinase activation pathway that increases sensitivity of call for insulin (Depta et al., 2022). T2D patients have less HNP 1-3 (neutrophil AMPs) and LL-37 compared to normal people. Few studies show that some AMPs using this type of case look promising. There are 45 AMPs have been identifiedwith antidiabetic activity. But only a few studies done on animal model till now. Nevertheless,tested peptides showed their anti-diabetic effect at lower concentration and they were not toxic to the cell. This gives a positive direction towards AMPs against diabetic complications (Deptaet al., 2022).

II) Role of AMPs role in diabetic foot infection

Diabetic mellitus complications with hyperglycemia leads to the development of diabetic neuropathy. Its symptoms include burning feet, pain, feeling abnormal cold, heat and results sensory never fibers damage. Also, injuries occur more easily as changes in foot shape occurs by motor neuropathy (Depta et al., 2022). DFS includes also ulceration of down part of ankle & SSTIs, bone infections. Microorganisms easily colonized in rupture skin of subcutaneous tissue and leads to infection. Lowes foot's arteries with atherosclerotic lesion leads to Ischemia which is a reason of non-healing wounds. Also, the drugs do not reach to the affected areas (Depta et al., 2022). Thus, antibiotic therapy also ineffective and most of the time bacterial biofilm forming during antibiotic therapy, it leads to the resistant to antibiotic. Several AMPs are effective against positive bacteria, negative bacteria, showing immunomodulatory & angiogenic property, neutralizing bacterial toxin, stimulating cells proliferation, migration, inhibiting biofilm formation, inhibiting pro-inflammatory. Thus, shows most effective result in wound healing (Depta et al., 2022).

High concentrated endogenous LL-37, combination of 1, 25 di-hydroxy vitamin D & L-isoleucine (increase Hbd-2 production) and LL-37 shows effectiveness in regeneration in DFU cell culture. Moreover, encapsulating LL-37 using nanoparticle lipid carrier, shows effectiveness in wound closure, reduction of inflammation of in-vitro & in-vivo (Depta et al., 2022). Furthermore, LL-37 with gold nano particle conjugation shows improvement of wound healing process compared to LL-37 using along. Another one, Temporin A and B not only improve wound healing process but also kills cells of *S.aureus*. AMPs like tachyplesin inhibits

resistant strains of *E.coli*, *S.aureous*, *C.neoformans*. Combination of nisin AMPs with pexiganan supplied by the bio gel, can inhibit and remove the biofilm formation which was done tested to a isolates diabetic foot infectious cell (Depta et al., 2022).

4.2. Therapeutic strategy for infected non healing wounds

From research it has been identified that both synthetic and natural AMPs significantly works for critical wound healing mechanisms by showing activity in cell migration, proliferation and in angiogenesis (Pfalzgraff et al., 2018). AMPs improves the critical condition of non-healing wound by boost up immune cells those will improve the healing process and by decreasing the pathogenicity factor induced production which causes inflammation (Pfalzgraff et al., 2018). There are some AMPs given with their mechanisms of action in table 3.

Table 3: Different sources of AMPs with their wound healing features (Wang et al., 2021).

AMPs	**Source**	**Wound healing mechanism**
Tylotoin	*Salamanders*	Shows immunomodulatory activity, promotes cell proliferation, migration, angiogenesis
AH90	*Odorrana grahami*	Stimulates TGF-beta secration, migration of keratinocytes
CW49	*Odorrana grahami*	Prevents excessive inflammation, proliferation
Temporins A and B	*Rana temporaria*	Keratinocytes proliferation and migration
Cathelicidin-Nv	Frog-*Nanorana-ventripunctata*	Keratinocytes proliferation, collagen productions in fibroblasts
LL-37	Human	Cell proliferation induction, migration & angiogenesis induction
DRGN-1	*Varanus komodoensis*	Re-epithelialization, granulation tissue formation

4.3. Uses of Cathelicidin-DM in wound healing process

Experiment on human proliferation using umbilical vein endothelial cells (HUVEC), skin fibroblast (HSF), immortalized keratinocytes (HaCaT) cell using CCK-8 assay with different concentrations of Cathelicidine-DM. This Cathelicidine-DM showed proliferation quickly in HSF, HUVEC, HaCaT cells in dose dependent manner (Wang et al., 2021). Using 10 μg/ml for HaCaT, the growth rate was 16.27% and using 20 μg/ml it showed 19.92% growth rate. Again, for HSF, the growth rate was 30.5% & 42.58% using 10 μg/ml & 20 μg/ml respectively. Also, for HUVEC cell it showed 107.32% & 177.17% for 10 μg/ml and 20 μg/ml respectively. Cathelicidine-DM significantly enhance the migration of cells. Moreover, mitogen activated protein kinase (MAPK) signaling pathway works for own healing mechanism and is related with cell proliferation, migration (Wang et al., 2021). Research has come up with that, Cathelicidine-DM can activate MAPK signal in pathway and provides wound healing functions. Cathelicidine-DM can activate phosphorylation of C-Jun N-terminal kinase (JNK), regulated protein kindness (ERK), mitogen activated protein kinase (P38) signaling pathway and this pathway has role in wound healing mechanisms (Wang et al., 2021).

4.4. Uses of hydrogel in diabetic wound healing

In recent study, it was found out that chitosan/ sodium-alginate (SA)/ velvet antler blood peptides (VBPS) hydrogel (CAVBPH) contains great antioxidant activity, low hemolysis rate. This compound contains desirable biochemical substance and can reduce inflammation. As diabetic wound healing mechanisms is a major clinical problem because of impaired angiogenesis and bacterial infection. And angiogenesis has a key role in wound healing mechanism as neovascularization is mainly responsible to transport nutrients, growth factors and can removes waste from the site of new tissue formation (Chen et al., 2021). So, this hydrogel is a great alternative therapy to promote angiogenesis and tissue regeneration.

4.5. AMPs in the clinical phase of development

From last few years, there are some clinical trials of AMPs drug formulation have been conducting. These formulations include hydrogel, topical cream, topical antibiotic and many

more. DPK-060, LTX-109, pexiganan, omiganan, gramicidin etc. AMPs medicines are now at clinical trial phase due to their potential and significant activity (Mahlapuu et al., 2020). DPK-060 is for the treatment of atopic dermatitis in the form of ointment, nano-capsule formulation, poloxamer gel formulation. 1% DPK-060 as ointment being examined for safety, efficacy using with polyethylene glycol and it is still now at clinical trial phase II (Mahlapuu et al., 2020). Also, omiganan for atopic dermatitis is in clinical trial phase III and it is in topical gel formulation. Recently another considered peptide is AES16-2M and this has been shown great improvement in atopic dermatitis and in wound healing process. Some selected AMPs in the clinical phase of development are shown in table 4 (Mahlapuu et al., 2020).

Table 4: AMPs in clinical phase of development (Mahlapuu et al., 2020).

Name of AMPs	**Source**	**Indication**	**Dosage form**
DPK-060	Human kininogen	Bacterial infection in atopic dermatitis	Ointment
LTX-109	Synthetic peptidomimetic	For impetigo	Topical gel
Pexiganan	African clawed frog	For infected diabetic foot ulcer	Topical cream
Omiganan	Bovine indolicidin	For atopic dermatitis	Injection

Chapter 5

Challenges and limitations of AMPs

Though, AMPs have shown great activity against a broad range of bacteria, fungi, viruses with various mechanism of actions and can be a great solution of bacterial resistance causing medication, good alternative of less effective treatment and in critical non-healing wound treatment. But it also has some limitations which need to be overcome as soon as possible. Firstly, AMPs can damage cell membrane of eukaryotes and can lead to hemolytic side effect. Secondly, there are rising production costing worldwide, it can be affected. Also, technical problems can limit AMPs manufactures. Moreover, AMPs certain stability is also limited to certain pH and environment (Huan et al., 2020). Furthermore, when AMPs apply to the biological system it can be denaturated and degraded by biomolecules including proteolytic enzyme, gastric environment this problem can occurs mainly when administered the medication orally (Roque-borda et al., 2021). In addition to, other shortcoming like Nano carrier aggregation non biodegradability also a challenge to overcome (Thapa et al., 2021). Future research is needed to report AMPs data in solving the associated problem on structurefunctional relationship (Huan et al., 2020). Several AMPs with hemolytic activity that is considered as important factor need to control and evaluate as because lysis of human cell occurs before AMPs can full filled the objectives (Roque-borda et al., 2021). Bacterial-resistance have been already reported against AMPs, it can also holding a risk to develop crossresistance with host AMPs or antibiotic therapy (Pfalzgraff et al., 2018). Moreover, only fewAMPs have been reached to treat skin diseases clinically including telavancin, daptomycin, oritavancin, guavanin 2, oritavancin etc. In addition to that, AMPs shows hemolytic activity which limits its clinical application. But some modification in their structure, combination with other potential compound and better understanding of AMPs mechanisms can lead to progress in the development of safe, effective drug that can be used clinically (Pfalzgraff et al., 2018).

Chapter 6

Future direction

To minimize the limitation of AMPs drug delivery, smart formulation method is applying by enhancing their chemical and metabolic stability (Mahlapuu et al., 2020). Chemical method of peptide mimetic and modification of synthetic amino acid have been applying to design AMPs to solve protease hydrolysis problem (Huan et al., 2020). For effective and safe delivery of AMPs, polymeric nanoparticles, different hybrid system can be applied (Thapa et al., 2021). Suitable ligands can be added with nanoparticle surface to target more specifically the infected sites. Moreover, covered with polymer or coating system can be applied in AMPs loaded nanoparticle for oral medication. So that, drugs those are micro-environment responsive or pH sensitive will be uncovered at target site and will exhibit effective in the microbial activity (Thapa et al., 2021). Furthermore, AMPs loaded nanoparticles can be conjugated with other nanoparticles to get proper targeted and controlled AMPs release (Thapa et al., 2021). Aggregation of nanoparticle can be prevented through suitable water-soluble polymer and surfactant. Silver and gold nanoparticles are getting interest for intrinsic antimicrobial effects. Also, their nanoparticles can be used as potential nano carrier for therapeutics including AMPs (Thapa et al., 2020).

Chapter 7

Conclusion

Irrational use of drug, and several ineffective resistance issues is occurring in recent decades. Different drug-resistant bacteria such as penicillin resistant, methicillin resistant, sulfonamide resistant, macrolide resistant, vancomycin-resistant, multidrug-resistant are identified and provides a serious challenge in the treatment of severe respiratory, topical, urinary & surgical site infections, bloodstream infection. In the search of new drug AMPs are getting interest and high priority. Due to various advantages including lower susceptibility to antimicrobial resistant, low toxicity, more selectivity towards target, several mechanisms of action against a broad range of pathogens, they can be a good alternative of conventional medication. AMPs can promote wound healing process by stimulating cell proliferation, migration and angiogenesis. AMPs can be a good choice for the treatment of atopic dermatitis, skin and soft tissue infections including diabetic foot infection and infected non-healing wound. And for these treatment Tylotoin, Temporin A and B, LL-37, Cathelicidin-DM, DPK-060, AES16-2M, Pexiganan etc have shown great improving activity. Though, there are some limitations and disadvantages but more research, clinical experiment and studies can eventually lead to development a smart, safe and effective AMPs delivery approach in the treatment.

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