1.1 INTRODUCTION

Everybody wants to lead a healthy life. Illness is a cause of mental trauma for the person as well as his family. Before the discovery of antibiotics even simple diseases proved to be fatal. Any type of infection in the body could lead to the death of the patient. Many diseases became treatable after the discovery and advancement in the field of antibiotics. Since many decades antibiotics have been a life saviour for all the people round the globe. Presently, there is an increase in the number of drug resistant bacteria. This is a cause of great concern as the diseases that are treatable till today will become again untreatable in future. Thus a need for alternate drugs arises. These alternate drugs should be developed in such a way that microorganisms find it difficult to develop resistance against them.

1.2 ANTIBIOTICS

In the history of medication, the antimicrobials are supposed to be leading form of therapeutics. The role that these antimicrobials have played in the control of severe disease causing microorganisms for the benefit of human race and thus in decreasing the rates of morbidity and mortality is quite remarkable [Aminov 2010]. The origin of the word antibiotic is from the word “antibiosis” having meaning “against life”. In the beginning, these antibiotics were thought to be poisonous natural compound that a microorganism produces against other microorganisms [Russell 1998]. Thus, an initially an antimicrobial was indicated by a substance of microorganism origin [Denyer et al. 2004] or naturally derived [Schlegel 1993]. These were considered to cause death of other microorganisms or cause hindrance to their development at low concentrations [Russell 1998]. With time, this description of antimicrobials became modified and its area was further expanded to include the antimicrobials that were partly or completely produced synthetically.

The antimicrobials against bacteria were divided into two groups- first which only inhibited the growth of bacteria, they were termed bacteriostatic and second were those which killed bacteria and were termed bactericidal [Walsh 2003]. Actually antibiotics are categorized based upon their action on various classes of microorganisms as antiviral, antifungal and antibacterial [Brooks et al. 2004; Russell 1998].

Late Sir Alexander Fleming, who was an English bacteriologist, discovered Penicillin in September 1928. Penicillin was the first antibiotic discovered. Penicillin was discovered accidently from *Penicillium notatum*. The reporting of this discovery was done in year 1929 [Aminov 2010]. Penicillin was first time tested on people in 1940 [Russell 1998; Schlegel 1993]. This discovery of Penicillin in 1920's and the beginning of its role in for the benefit of mankind in 1940's and its further development and transformation to combat bacterial infections has continued [White and Cox, 2013].

It is known that very helpful microorganisms are present in our gastrointestinal tract; these antibiotics can act against both pathogenic microorganisms as well as these useful microorganisms; because these are not selective in nature [Walsh 2003; Etebu et. al. 2016].

1.3 MECHANISM OF ACTION OF CONVENTIONAL ANTIBIOTICS

The mechanism of action of conventional antibiotics is mostly dependent upon the structure of bacteria like their bacterial membrane composition etc. or the various metabolic paths followed within the microorganisms. The various mechanisms of action followed by conventional antibiotics are as follows -

- Mechanism of action via inhibiting the synthesis of cell wall
- Mechanism of action via inhibiting the function and structure of nucleic acids
- Mechanism of action via inhibiting protein synthesis

- Mechanism of action via blocking the key metabolic pathways

  [Talaro and Chess 2008; Madigan and Martinko 2006; Wright 2010]

1.3.1 Inhibition of cell wall synthesis

Almost all bacterial cells composed of an inflexible layer of peptidoglycan (PG), also called as murein earlier times. Peptidoglycans are involving in the protection of cells by prevailing osmotic pressure. This protection is needed by bacteria to exist in harsh environmental conditions [Bugg and Walsh 1992; Holtje 1998]. Peptidoglycans are necessary for bacteria to remain alive, PBPs such as Transglycosylases and Transpeptidases help in the synthesis of Peptidoglycan. Both these enzymes have crucial role in the synthesis of peptidoglycan. These play a role in cross-linking immature peptidoglycan strands and also extension of the peptidoglycan strand by adding disaccharide pentapeptide to the glycan strands [Park and Uehara 2008]. Antibiotics like cephalosporins, carbapenems and penicillins [Josephine et al., 2004], antibiotics belonging to the class glycopeptide like vancomycin [Kahne et al. 2005; Etebu Arikekpar 2016], etc. have this mechanism of action.

1.3.2 Inhibition of nucleic acid synthesis

Nucleic acid synthesis is very essentials for the survival and posterity of bacterial cells and the antibiotics which are able to disrupt the synthetic pathways of the nucleic acids are important antimicrobials. Such antimicrobials either block transcription or replication process as these inhibit synthesis of nucleic acid. Helicases are one of the enzymes which are targeted by such antibiotics [Gale et al. 1981]. The other two enzymes which are a target in the inhibition mechanism by nucleic acid synthesis inhibiting antibiotics are Topoisomerase II and Topiisomerase IV. The synthesis of RNA is inhibited by such antibiotics as these affect the activities of the above two enzymes by negatively affecting the action of RNA polymerase. Quinolones group of antibiotics have this type of mechanism of action [Gale et

al. 1981, Chen et al. 1996]. These are specific in their mechanism of action against bacteria by inhibiting the synthesis of nucleic acids; as these quinolones do not affect the RNA polymerase of mammals. These act against some Gram-negative bacteria and also on Gram-positive bacteria [Etebu and Ibemologi 2016].

1.3.3 Inhibition of protein synthesis

The type of protein produced by a bacterial cell is determined by the information carried on the codons of the DNA, which the DNA passes on to the mRNA (messenger RNA). The types and amount of proteins produced by all the living organisms characterises them as proteins are the biomolecules responsible for the structure and function of organisms. The amount and type of protein present in the microorganism is governed by the information carried on its DNA. The information on the DNA is further passed on to mRNA, which along with tRNA (transfer RNA) is involved in translation of proteins. mRNA and tRNA move to ribosomes, where protein translation occurs and the amino acids, specific for the codons, build up the protein molecule [Etebu 2013]. The translation of proteins is carried out in three steps – Initiation, Elongation and Termination. Several cytoplasmic accessory factors and ribosomes play a role in protein translation [Gualerzi et. al. 2000]. Ribosomes comprise of a RNA and proteins and thus are referred to as ribonucleoprotein. The RNA component of Ribonucleoprotein consists of two subunits- large subunit 50S and small subunit 30S. This RNA component is known as Ribosomal RNA (rRNA) [Nissen et. al. 2000]. The three genes present on the rRNA of bacteria are 5S, 16S and 23S [Moore 2001; Lafontaine and Tollervey 2001]. The scientists have made use of the characteristic difference between the eukaryotic and prokaryotic rRNA, for the development of antibiotics specific for several pathogenic bacteria [Hong et. al. 2014]. Because of so many important functions of proteins in the living beings, these are an important drug target. These drugs act by either inactivating the cells;

completely killing it or inhibiting the growth. Linezolid, chloramphenicol, lincomycin, clindamycin, erythromycin etc [Douthwaite 1992; Katz and Ashley 2005; Patel et al. 2001; Vannuffel and Cocito 1996; Menninger and Otto 1982], Oxazolidinones [Patel et. al. 2001], macrolides like streptogramin and lincosamide [Vannuffel and Cocito 1996; Menninger and Otto 1982], spectinomycin, streptomycin, tetracycline, etc. [Hong et al., 2014; Chopra and Roberts, 2001; Epe and Woolley 1984], aminoglycosides, spectinomycin, streptogramins, etc. belong to this category. Chloramphenicol, mainly shows bacteriostatic behaviour can also be bactericidal against *Neisseria meningitides* and *S. pneumoniae* [Rahal and Simberkoff 1979] and also against *H. influenza* [Rahal and Simberkoff 1979; Goldstein et al. 1990]. This difference of the mechanism of action from species to species is due to the presence of variations in the conserved RNAs and ribosomal proteins [Roberts et al., 2008; Etebu Arikekpar 2016].

1.3.4 Blockage of key metabolic pathways

Enzymes of the metabolic pathways are the important targets for the development of novel drugs. Several antibiotics mimics the substrate for the particular enzyme hence blocking the enzyme activity and ultimately kill the bacteria as these enzymes needed for the important cellular metabolism of the bacteria, Examples are sulphonamides and trimethoprim. Bacterial cells require tetrahydrofolate for folic acid synthesis. The antibiotic sulphonamide mimics this tetrahydrofolate [Talaro and Chess 2008]. Folic acid is essential for the metabolism of amino acids and nucleic acids. By mimicking the structure of tetrahydrofolate, sulphonamide inhibits the production of amino acids and nucleic acid (RNA and DNA) [Talaro and Chess 2008; Etebu Arikekpar 2016].

1.4 ANTIBIOTIC RESISTANCE

The history of antibiotic resistance genes can be uncovered through the phylogenetic analysis which proposes the long-term presence of genes conferring resistance to several classes of antibiotics in nature long time before the antibiotic era [Aminov 2010]. Antimicrobial resistance is neglected and never been a priority area in most developing and in many developed countries. Despite the fact that resistance against antibiotics is at abnormal states in numerous places in India, the issue remains to a great extent obscure in light of the fact that there is very little published report and relatively lack of a surveillance system is present. The issue of antibiotic resistance came into light only when New Delhi metallo-β-lactamase-1 (NDM1) was first reported in 2009 [Global Antibiotic Resistance Partnership 2011]. NDM1 is an enzyme produced by the gene *blaNDM-1,* carried on plasmids, could be transferred to many bacterial species, for example *Klebsiella pneumoniae* and *Escherichia coli*, conferring resistance to multiple antibiotics, including carbapenems [Global Antibiotic Resistance Partnership 2011; Choudhury et. al. 2012]. Antibiotic resistance is probably linked with the uncontrolled and irrational used of antibiotics and once resistance appears, there is a certain time period, which varies considerably with the antibiotic in question, until the resistance becomes prevalent enough to alter the perceptions of medical practitioners [Shlaes and Projan 2009]. In India, there is enormous and growing problem of antibiotic use and abuse in neonatal care and unless neonatologists stop using broad spectrum antibiotics for prolonged periods, resistance to antibiotics will rise [Isaacs 2005]. Use of antibiotics is not limited to therapeutics that accounts only less than half of antibiotics produced commercially, but also as growth promoters in animal farms, aquaculture and as anti-coccidial agent in poultry besides their use in research, industry, biocides in hand care and household cleaning products [Davies and Davies 2010]. Lack of rapid and easy diagnostics is another factor contributing to injudicious use of antibiotics [Ganguly et al. 2011]. Easy and rapid diagnosis of causative

organism and their antibiotic susceptibility pattern will help physician to choose targeted therapies [Acar et. al. 2009]. An important interventional measure is the dose/duration of antibiotic treatment and careful selection of dosing regimens based on pharmacodynamic and pharmacokinetic properties of drugs will prevent emergence of antibiotic resistance and selection of mutants (mutant prevention concentration) [DeRyke et. al. 2006]. Emergence of resistance to new drugs is now be noted in the developed world and hence they take proper care and also involves to discovers new drugs well before the condition worsen and uncontrollable . However, the ARGs (antibiotic resistance genes) are rapidly spreading in the developing world due to the rapid urbanisation without proper sanitation and hence contributing in the emergence and dissemination of antibiotic resistant bacteria. Sahoo *et al*. [Sahoo et. al. 2012]. The study clearly pointed out the positive impact of proper healthcare facilities available to healthcare practitioners, sanitation and public awareness on the transmission of ARGs. India has the largest number of private sector physicians in the world, often these practitioners have dubious qualifications and unregulated prescribing practices [Udwadia et. al. 2012]. Intensive care units (ICUs) in the hospital are the hot-spots of ARG transmission. Diwan *et al.* [Diwan et. al. 2010] quantified antibiotic residues in the waste waters associated with a hospital in India and assessed their association with quantities of antibiotics prescribed in the hospital and the susceptibility of *E. coli* found in the hospital effluent. There is a direct correlation was observed between the isolates from two sources highlighting the need of further research in this area. To reduce the burden of antibiotic resistance, it is advised to the hospitals that they must treat hospital waste before its release into the environment [Acar et. al. 2009]. It has been demonstrated that the rates of hospital-acquired bacterial infection and recurrence of antibiotic resistance can be lessened by diminishing the rate of turnover of patients, applying the transmission control measures and the utilization of second-line drugs for which there is no resistance [Haber et. al. 2010].

NDM-1, a recently described metallo-beta-lactamase (MBL), have a place with the group of carbapenemases, was first identified in single isolates of K. pneumoniae and E. coli, both recuperated from a patient repatriated to Sweden after treatment in a hospital in New Delhi, India [Ganguly et. al. 2011]. Subsequent studies reported NDM-1 from a centre in Mumbai, following isolation of MDR Enterobacteriacae in clinics in Chennai and Haryana and from drinking water and waste water in New Delhi. NDM-1 might be the most generally known type of antibiotic resistance in India [Choudhary et. al. 2012].

In the 21st century, the pharmaceutical business is encountering dramatic changes. Stringent safety regulations, lengthy compound development processes and monstrous budgetary endeavours (Vlieghe et al. 2010) all incur concern that, in spite of the expanding investment research and development, medicinal innovation is declining. Particularly the last decade has seen a noteworthy change in the scope of the pharmaceutical sector, concentrating more on orphan or repurposed medications and decreasing generation costs, as to persevere through the high costs related with drug development. Less new medications make it to the market and the patent insurance of current blockbuster drugs is falling apart, with a resulting drainage of the drug pipelines. This may at last push the pharmaceutical business towards another frontier in modern drug development. Fresh techniques are needed to restore pharma's lost momentum and we concur with Vlieghe and co-authors (Vlieghe et al. 2010) that the sector’s hope (partly) lies in peptides [Uhlig et al. 2014].

1.5 ANTIMICROBIAL PEPTIDES

Global increase in microbial resistance against conventional antibiotics, there is a need to search alternatives against the microbes and the antimicrobial peptides might be a good candidate for the same [Uhlig et al. 2014].

Several biological active peptides harbours virtually all living species therefore it represents one of the most promising sources for peptide drug discovery. Naturally occurring peptides exerts variety of biological roles most importantly as signalling/regulatory molecules in a broad variety of physiological processes, including defence, immunity, stress, growth, homeostasis, and reproduction [Hancock and Sahl 2006]. Peptide evolution leads to acquire the ability to exhibit their 'natural' bioactivity outside of the producing organism such as many peptides are isolated and characterised from the skin of frogs and toads [Pukala et. al. 2006; Shaw 2009]. These genetically encoded peptides involve in the defence mechanism of manufacturers against many foes, both predators and pathogens [Epand and Vogel 1999]. Until now almost 300 antimicrobial peptides have been identified from amphibians which hold promise in the antibiotic research and development [Li et al. 2007]. Intriguingly, numerous remotely active peptides have developed as methods for active predation, particularly in venomous creatures, for example, spiders, snails and snakes [Vetter et al. 2011]. While the toxicity emerges from meddling with neuronal transmission (blocking synaptic signalling, ion channel; e.g. conotoxins) or, when all is said in done, upsetting critical biochemical signalling systems inside the prey's body [Tennessen 2005], low dosages of these peptides can really neutralize aggravations from differing disorders. In like manner, harmful peptides may help in treating pain [Miljanich 2004], neurological and cardiovascular illnesses, diabetes and cancer [Tennessen 2005; Lewis and Garcia 2003]. An eminent example is the type 2 diabetes medicate Exenatide, a synthetic version of a glucagon-likepeptide-1analogue found in the venom of the Gilamonster *Heloderma suspectum* [Bond 2006]. As bioactive peptides got from natural sources have been liable to ages of selective pressure, they indicate significant plusses over artificially/chemically conceived peptide-like compounds. Such as, they exceed expectations in stability and target affinity, both of which are greatly challenging to accomplish or imitate through rational peptide design, screening of

libraries of arbitrarily made peptides or peptidomimetics. Despite the fact that we welcome the insight of peptide medicinal chemists, and other customary (bio)chemistry based pharmacologists, we believe that much is still to be discovered from the natural bioactive peptides utilized everywhere throughout the biological taxonomy (from microorganisms over plants to animals).With such a significant number of these being utilized as medications by such a large number of various species for such a large number of various purposes, plainly humanity can at present take in a great deal from the inferred science [Uhlig et al. 2014].

1.6 CLASSIFICATION OF ANTIMICROBIAL PEPTIDES

Antimicrobial peptides classified in two groups on the basis of charge as anionic antimicrobial peptides (AAMP) and cationic antimicrobial peptides (CAMP) [Zucca et al. 2011; Brogden 2005]. The AAMPs are negatively charged with a charge ranging from -1 to -7 [Zucca et al. 2011] as they have large number of negatively charged amino acids which give them a net negative charge. The AAMPs are killing the microorganisms through some unidentified mechanism. Maximin H5 and Dermcidin examples of AAMP found in amphibians and humans respectively [Brogden 2005]. The CAMPs are positively charged with charges ranging from +2 to +11 [Zucca et al. 2011] having plenty of positively charged amino acids like lysine, histidine and arginine are accountable for the net positive charge of these peptides. These positively charged peptides have a strong electrostatic attraction towards the contrarily charged cell membrane surface of the bacterial membranes. The eukaryotic cell membrane is neutral charged on its surface [Zasloff 2002]; hence these have weak attraction towards it. Due to this behaviour of CAMP, it only gets attracted towards the prokaryotic cell membrane rather than eukaryotic cell membrane. CAMPs also have the higher percentage of hydrophobic residues which helps them to penetrate the cell membrane. Thus, the chances of CAMP being harmful to the host are minimized that’s why the medical

practitioners and the scientists are working to develop antimicrobial peptides as an alternative for the conventional antibiotics to combat the menace of drug resistant bacteria. CAMPs are further classified in the α-helical cationic antimicrobial peptide, β-sheet cationic antimicrobial peptide, linear cationic antimicrobial peptide and loop structure cationic antimicrobial peptide based on their structures. The first two are found commonly in nature [Giuliani et al. 2007]. The α-helical cationic antimicrobial peptides like LL-37, cecropins, magainin's. do not possess any disulphide bridges as they lack cysteine. The β- sheet CAMPs, e.g. Human α and β defensins, plectasin, protegrins, possess two to five disulphide bridges. The linear CAMPs such as Indolicidin are rich in glycine, proline, tryptophan, arginine and/or histidine. The loop structure CAMPs such as Bactenecin and thanatin possess one disulphide bridge [Ojha et al. 2013].

1.7 MODE OF ACTION OF ANTIMICROBIAL PEPTIDES

Bacterial membranes having large number of negatively charged lipids having phospholipid head groups, for example, phosphatidylglycerol, cardiolipin and phosphatidylserine, whereas the mammalian membranes are rich in zwitterionic phospholipids, like phosphatidylethanolamine, phosphatidylcholine and sphingomyelin [Yeamen and Yount 2003]. Thus, the positively charged CAMP show a high electrostatic attraction for the negatively charged bacterial membrane whereas weak electrostatic attraction for mammalian membrane which is neutral charged. The CAMP neutralises the bacterial cells by either disrupting their cell membrane or by targeting the intracellular targets which inhibits the essential processes required for the survival of microorganisms. The mode of action of CAMPs against the bacteria is based on the net negative charge, hydrophobic nature and the amphiphilic nature [Keymanesh 2009]. CAMPs are initially attracted towards the bacterial membrane due to the electrostatic attraction and this attraction resulting in to the

accumulation on the bacterial membrane surface and when the threshold accumulation reached the antimicrobial peptides start penetrating inside the bacterial membrane where they attack the intracellular targets.

1.7.1. Penetration of CAMPs in the cell membrane

There are three mechanisms by which the CAMPs penetrate the cell membrane - Barrel-Stave Mechanism Toroid Pore or Wormhole Mechanism and Carpet Mechanism [Brogden 2005; Yeamen and Yount 2003; Cézard et al. 2011].

1.7.1.1. The Barrel-Stave Mechanism:

The peptides forms barrel like ring around the aqueous pore where the hydrophobic surfaces of the CAMPs face towards the outside of the pore (where they come in contact with the acyl chains of the membrane) and the hydrophilic surfaces form the inner lining of the pore. The positively charged amino acids are placed near the phospholipid head groups. The peptides undergo a conformational change when they bind to the surface of bacterial cell membrane. In this process the polar head groups of the membrane move aside thus causing membrane thinning and the hydrophobic surface of the CAMP penetrates here. As the number of bound peptides reach a threshold concentration then the CAMPs aggregate. This aggregation causes pore to expand and the CAMPs traverse across the membrane [Brogden 2005; Yeamen and Yount 2003; Cézard et al. 2011]..

1.7.1.2. Toroid Pore or Wormhole Mechanism:

Toroid Pore model of the penetration involves the intercalation of CAMPs with the bacterial cell membrane and acquire α- helical configuration. In this model the polar region of the CAMPs and the polar head group of the lipids in the membrane interact with each other. The CAMPS align themselves perpendicular to the membrane and its hydrophobic regions

interact with the hydrophobic tails of the lipids in membrane forming a transient toroidal pore complex. Some peptides are transferred to the cytoplasm when these pores disintegrate [Brogden 2005; Yeamen and Yount 2003; Cézard et al. 2011]..

1.7.1.3. The Carpet Mechanism:

The CAMPs accumulate parallelly on the bacterial membrane surface, like a carpet, in high density and when a threshold concentration is reached then the membrane integrity is lost due to unfavourable energetic. This concentrated layer of CAMPs on the surface causes destabilization of phospholipid packing and thus leads to membrane disruption [Brogden 2005; Yeamen and Yount 2003; Cézard et al. 2011]..

1.7.1.4. Mechanism of Cell death

The cationic antimicrobial peptides follow different systems for causing cell death. The CAMPs connect with the cell membrane and causes its dysfunction. These CAMPS can enter inside the cell and they can hinder the synthesis of extracellular biopolymers or they can restrain the intracellular functions.

The cell membranes play very important role in many cellular functions, e.g. maintenance of gradients, cellular energetic, selective permeability, etc. Membrane dysfunction can restrict with some of these critical functions and thus kill the bacteria either directly or indirectly. The membrane disruption can cause seepage of ions or metabolites, depolarization of membrane, etc., which can lead to the cell death. Antimicrobial peptides interact independently with the outer and inner membrane of gram- negative bacteria. When gram-positive cells are exposed to antimicrobial peptides, then immediate increase in water and ion flow causes swelling and osmotic dysregulation. Thus, the cell membrane dysfunction can cause a major role in leading to cell death due to the action of CAMP.

The CAMPs can also inhibit the biosynthesis of some extracellular macromolecules like peptidoglycan, chitin etc. as their synthesis is dependent on the cell membrane integrity. Thus, loss of membrane integrity inhibits their synthesis, leading ultimately to cell death.

There are a few situations where the cells survive for some time after membrane permeabilization and afterward cell death happens so in these cases it can be presumed that the cell death happened not by membrane dysfunction but rather some other reason. These CAMPs kill the microorganisms by disturbing some intracellular functions like hindrance of DNA, RNA or peptide synthesis, etc. [[Brogden 2005; Yeamen and Yount 2003; Cézard et al. 2011].

1.7.2 Mode of action of AAMPS

AAMPs bind to microbial membranes through variety of mechanisms AAMPs that include cationic residues often use these residues to bind directly to anionic components of target membranes. Those AAMPs which are devoid of cationic residues the membrane binding of these peptides appears to be facilitated by the presence of cationic metal ions. In the AAMPs where no anionic residues and as well as no cations the membrane binding appears to be principally hydrophobicity driven. Membrane binding by AAMPs prompts the utilization of a differing array of antimicrobial mechanisms. At times, these AAMPs translocate over the microbial membrane to use intracellular sites of action while in different cases, this membrane itself is the principle site of action utilized by these peptides. In the latter case, AAMPs can actuate microbial cell death by means of various membrane related impacts, including permeabilization of the bilayer through pore formation and the disintegration of membrane integrity using a carpet-type mechanism. It appears to be likely that the moderately non-specific nature of these membrane based mechanism of microbial inactivation contributes to the fact that microbial resistance to the action of AAMPs has a

very low incidence [Harris et al. 2009]. Most significant mechanisms of resistance to the action of conventional antibiotics derive from spontaneous mutations in the microbial genome, which prompt changes to the specific sites of activity that are focused by these anti-microbials [Davies and Davies 2010]. In any case, it appears to be unrealistic that microorganisms would get the large number of cumulative genetic changes potentially needed to inhibit the mechanisms of membrane invasion utilized by AAMPs in their antimicrobial action. Besides, given the location of this site of action, it would appear to be impossible that the movement of AAMPs would be influenced by the activity of efflux pumps, a mechanism utilized by numerous multi-drug resistant microbial pathogens to expel conventional antibiotics [Davies and Davies 2010; Harris et al. 2011].

1.8 THERAPEUTIC POTENTIAL OF ANTIMICROBIAL PEPTIDE

Naturally occurring AMPs have enough scope to act as antibacterial agent alone or in combination with traditional antibiotics. Therapeutic potential of antimicrobial peptides has been studied by different groups of workers. Several cationic antimicrobial peptides are being tested for their efficiency as antimicrobial agents and found to possess broad spectrum of activity. Peptides from the seed and pod of *Pisum sativum L*. were found to be active against bacteria [Rehman and Khanum 2011]. Fresh aqueous extract of garlic showed inhibition of growth for some *Salmonella serovars* [Belguith et al. 2010]. BEP-1 isolated from *Bullacta exarata* showed activity against human pathogen strains such as *Staphylococcus epidermis*, *E. coli* and Methecillin-Resistant *Staphylococcus aureus* [Jian-yin et al. 2011].

The microorganisms have less probability to develop resistance against these CAMPs peptides on excessive use. This is because the antimicrobial peptides are short peptides which interact with the bacterial membrane. To develop resistance a large part of the bacterial

membrane needs to be modified, which is a very energy consuming process [Baltzer and Brown 2011].

Generally, the antimicrobial peptides are found non-toxic for the eukaryotes as they are specific to get attracted towards the prokaryotes. Several antimicrobial peptide drugs are being tested; some are in the early phase of development (phase I, II and preclinical) while some are in the late clinical stage (phase III and advance). Only few of them have crossed all these barriers to reach the market [Guilani et al. 2007]. There are some problems in the path of developing antimicrobial peptides as drugs particularly because these antimicrobial peptides are susceptible to proteases, change in pH, etc. [Seo et al. 2012, Lee et al. 1997]. Also, the cost of production of antimicrobial peptides is comparatively higher than conventional antibiotics [Cézard et al. 2012, Seo et al. 2012].

Studies are being done to handle all the previously mentioned issues through various methodologies. The AMPs are mostly found in the L- configuration in nature, which are inclined to be degraded by the proteases and subsequently rendered innocuous for the pathogens. To overcome this challenge, the antimicrobial peptides are orchestrated in their D-form, which is unnatural and the proteases can't degrade them as it isn't their substrate for activity [Seo et al. 2012]. A few properties of an antimicrobial peptide D1 (K13) were changed, which prompt change in its activity against gram negative bacteria [Jiang et al. 2011]. A few endeavours are being completed to create antimicrobial peptides as medications which are less harmful with no trade off with their antimicrobial action. Work is being done to plan antimicrobial peptides to fill in as a substitute of traditional anti-microbials. The best method, proposed for studying the biological activities of AMPs is to study the synthetic mimetic of AMPs (SMAMPs) [Som et al. 2008]. QSAR modelling based on physicochemical properties of cationic AMPs can fill in as useful tool in peptide design [Taboureau 2010;

Wang et al. 2012] and development of suitable AMPs with upgraded activity and decreased danger.

1.9 TOXINS USED IN THE PRESENT STUDIES

In my work I have studied the *in silico* interactions between various toxins and antimicrobial peptides. The following toxins were used in my studies:

1.9.1 ETA, ETB and PVL toxins of *Staphylococcus aureus*

1.9.1.1 Selection criteria

Two exfoliative toxins (ETA and ETB) are responsible for the staphylococcal scalded skin syndrome (SSSS) [Ladhani 2003]. Human infecting strains of *S. aureus* produce ETA and ETB [Bukowski 2010]. PVL is associated with necrotizing skin infections. It belongs to subfamily of pore-forming toxins. PVL provides *S. aureus* high virulence and protection against the host's immune system.

1.9.1.2 Structure

The ETA (FIGURE 1.1; pdb id: 1DUA) is heat stable and located on chromosome. The ETA consists of 242 amino acids and has molecular mass of 26,950 Da. The ETB is heat labile and located on plasmid. The ETB (FIGURE 1.2; pdb id: 1DT2) consists of 246 amino acids and has molecular mass of 27,274 Da [Ladant et al 2005]. The crucial residues of ETA are His72, Asp120 and Ser195 [Rago et al 2000]. The residues essential for the functionality of ETB are His65, Asp114, Ser186 and Ser202 [Vath et al 1999]. The RCSB pdb id of ETA and ETB are 1DUA and 1DT2 respectively. The crucial residues of PVL are Asp43, Ile59, Phe76, Trp78, Gly79, Tyr82, Tyr99, Ala100, Pro101, Trp164, Met192, Phe193, Phe207, Leu216, Ser217, Phe221, Pro223, Phe225, Ile226, Tyr245, Arg247, Asp250, Tyr252, Asn265

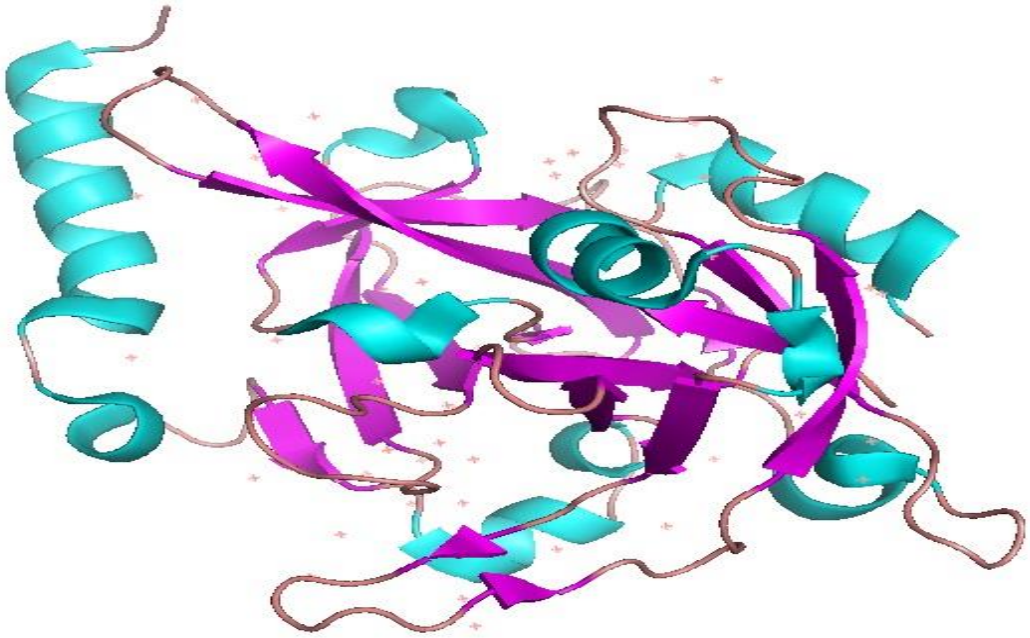

FIGURE 1.1: RCSB downloaded shows 3-D structure of Exfoliative toxin A (1DUA)

and residues from 103-153 [Pédelacq et al 1999]. The RCSB pdb id of PVL is 1PVL (FIGURE 1.3).

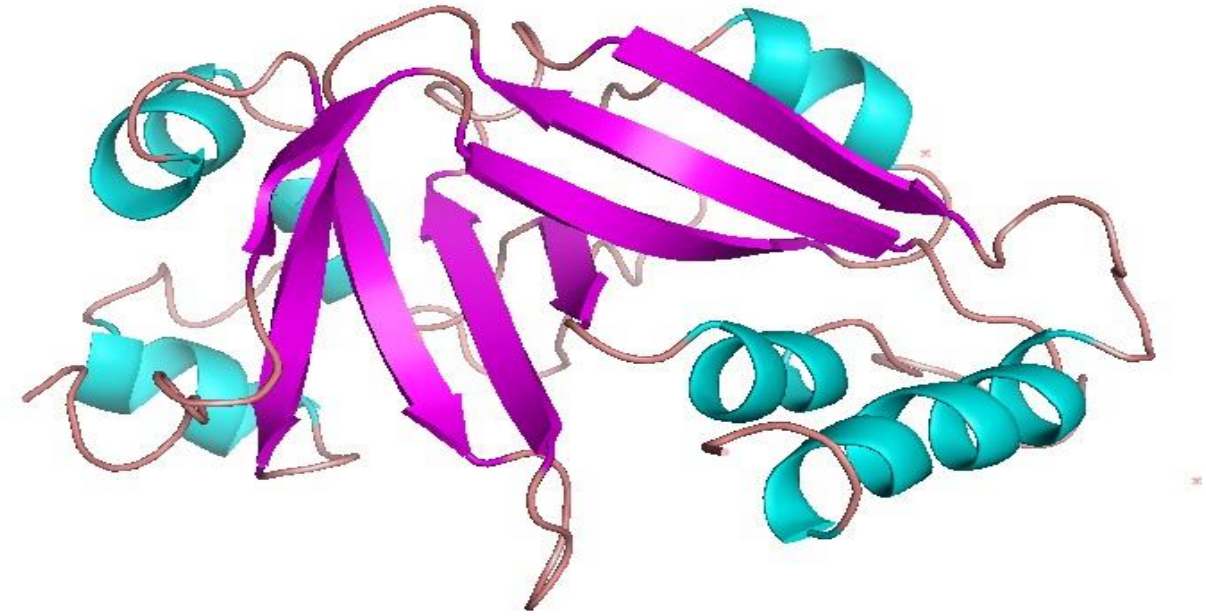

FIGURE 1.2: RCSB downloaded 3-D structure of Exfoliative toxin B (1DT2)

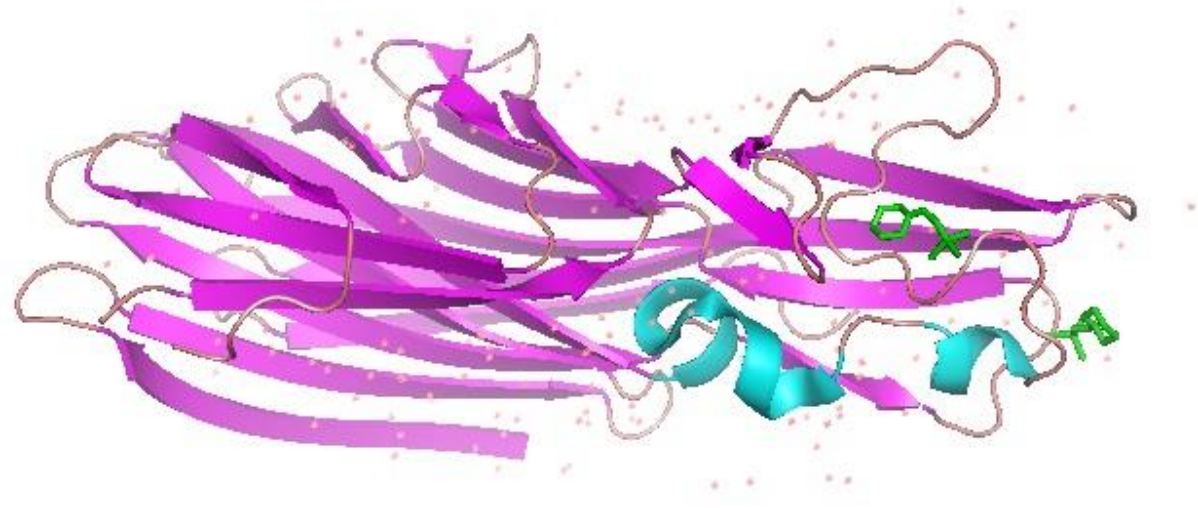

FIGURE 1.3: RCSB downloaded 3-D structure of Panton Valentine Leukocidin (1PVL) with ligand 2-(n-morpholino)-ethanesulfonic acid.

1.9.2 Transcriptional activator ToxT of *Vibrio cholera*

1.9.2.1 Selection criteria

Vibrio cholerae is the causative agent of a dreadful intestinal infection Cholera. Two virulence agents responsible for causing the disease are produced by V. cholerae. These are cholera toxin (CT) and Toxin-coregulated pilus (TCP). A transcriptional cascade controls the expression of these virulence agents. ToxT, an AraC family regulator, is the culminating point of the transcriptional cascade. The ctx and tcp operons are controlled by promoters. ToxT binds to tox boxes and thus activates transcription of these operon promoters. Tox boxes are thirteen base-pair sequences. The structure of the promoter is responsible whether ToxT binds as a monomer or dimer. The Tox box sequences can be either present in inverted or direct repeats, or in single configuration, so that ToxT can play its role in the positive regulation of gene transcription [Lowden et al. 2010].

1.9.2.2 Structure

The 3D structure of ToxT was used for our study. This was downloaded from RCSB database. The pdb id of this structure was 3GBG (FIGURE 1.4, FIGURE 1.5). The structure consists of nine beta-strands and ten alpha-helices. This 3D structure has missing residues in it. No coordinates were determined for the region from amino acids 100-109. The region of beta-strands in the 3D structure 7-14, 19-26, 31-35, 40-44, 49-53, 57-61, 66-73, 79-86 and 114-118. The region of alpha-helices in the 3D structure 88-97, 123-131, 141-155, 170-180, 188-195, 199-207, 213-231, 236-242, 248-259 and 263-269. Palmitoleic acid is the ligand attached with ToxT in this structure.

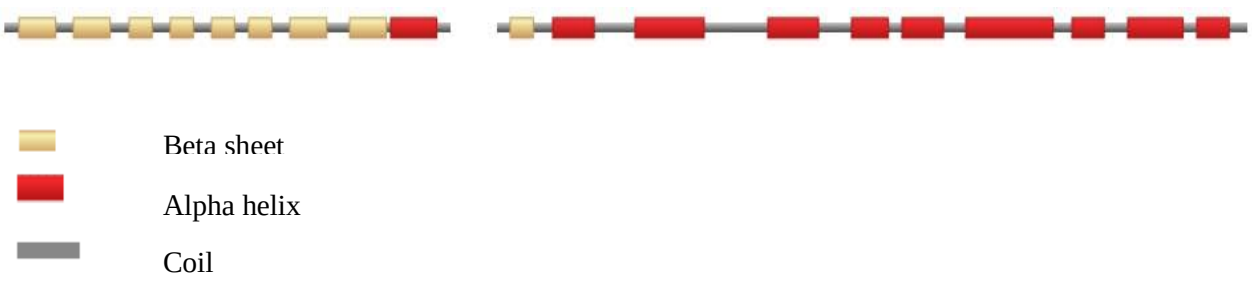

FIGURE 1.4: The figure shows the secondary structure of 3GBG

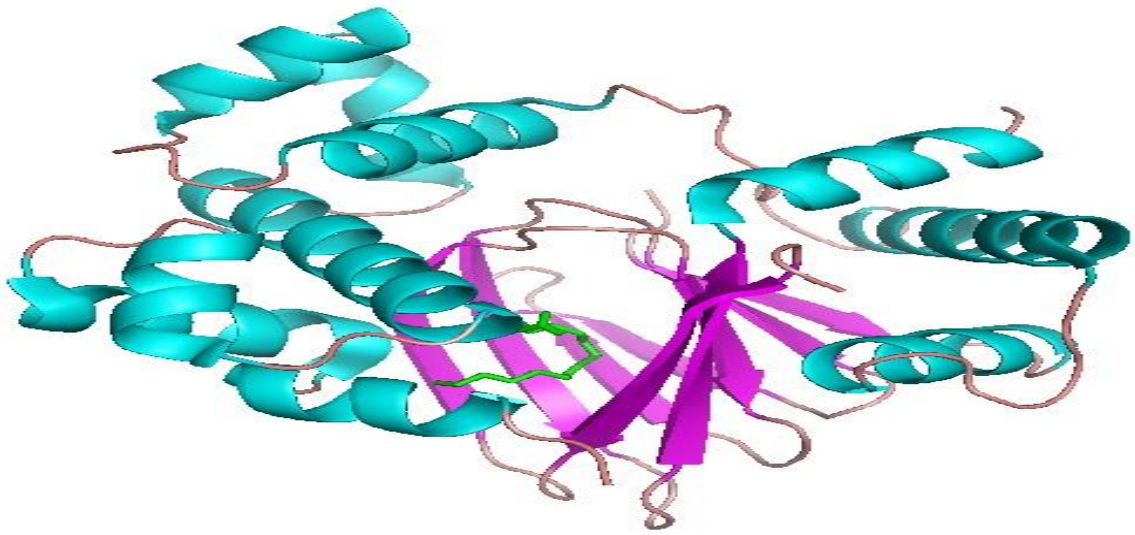

FIGURE 1.5: RCSB downloaded 3D structure of 3GBG with ToxT and Palmitoleic acid

ToxT stability and folding depends upon many N-terminal hydrophobic core residues. These residues are Met32, Trp34, Ile35, Leu42, Leu60, Leu71, Trp117, Leu127, Phe147, Phe148, Phe151 and Phe152. Glu52 residue of β5 sheet and Glu129 residue of α2 are exposed on surface and Ser140 residue are also important for function of ToxT. The C-terminal residues crucial for stability and folding of ToxT lie in the cores of Helix-turn-helix1 (HTH1) and Helix-turn-helix2 (HTH2). These residues include Ile174, Val178, Trp186, Trp188, Leu206, Val211, Ile217, Phe245, Phe251 and Phe255. Some residues exposed on the surface of ToxT are crucial for the stability of DNA binding domain (DBD). These residues include Ser175, Arg184, Arg221, Ser227, Glu233, Lys237, Gly244 and Asn260. Residues required for ToxT and DNA interactions are Lys203 residue of α6, Arg214 residue of α7, Thr253 residue of α9 and Ser257 residue of α9 [Childers et al. 2007].

1.9.3 Diphtheria toxin of *Corynebacterium diphtheriae*

1.9.3.1 Selection criteria

Corynebacterium diphtheriae is a pathogenic bacterium, which secretes an exotoxin, Diphtheria toxin. Diphtheria toxin hinders the protein synthesis machinery of eukaryotes. The component of protein synthesis machinery which is sensitive for Diphtheria toxin is elongation factor-2 (EF-2). The EF-2 is a polypeptide chain elongation factor during protein synthesis in eukaryotes [Collier 1975]. Diphtheria toxin is a member of the A-B toxin family. Fragment A is the N-terminal fragment which is responsible for the ADP-ribosyl transferase function of Diphtheria toxin. Fragment B is the C-terminal fragment and it functions in binding the toxin to the receptor on the cell. Reduction of the disulphide and proteolysis of the peptide bonds separated the two fragments, as these fragments are joined by disulphide bridges and peptide bonds. The separation of fragments is necessary for the Diphtheria toxin to cause toxicity [Collier 2001].

1.9.3.2 Structure

The catalytic domain of the diphtheria toxin (FIGURE 1.6; pdb id: 1DTP) interacts with NAD to cause toxicity. It has been predicted that the adenosine ribose in NAD+ is near the His21 residue of diphtheria toxin [Papini et al. 1989]. The Tyr65 residue of the diphtheria toxin is found at the NAD binding site [Papini et al. 1991]. The residue that is important for the catalysis role of Diphtheria toxin for catalyzing the ADP-ribosylation of the EF-2 is Glu148 [Wilson et al. 1990; Carroll et al. 1985]. Lys24 forms a salt bridge between with the phosphate group in NAD [Bennett et al. 1994]. Tyr54 and Tyr65 form a groove in which Nicotinamide ring and Nicotinamide mononucleotide (NMN) ribose fit into. The residues 39-46 form an active site loop. The residues Tyr27, Ile31, Ile35, Pro38, Phe53 and Trp153 form a hydrophobic pocket in which the adenine ring binds [Bell and Eisenberg 1996].

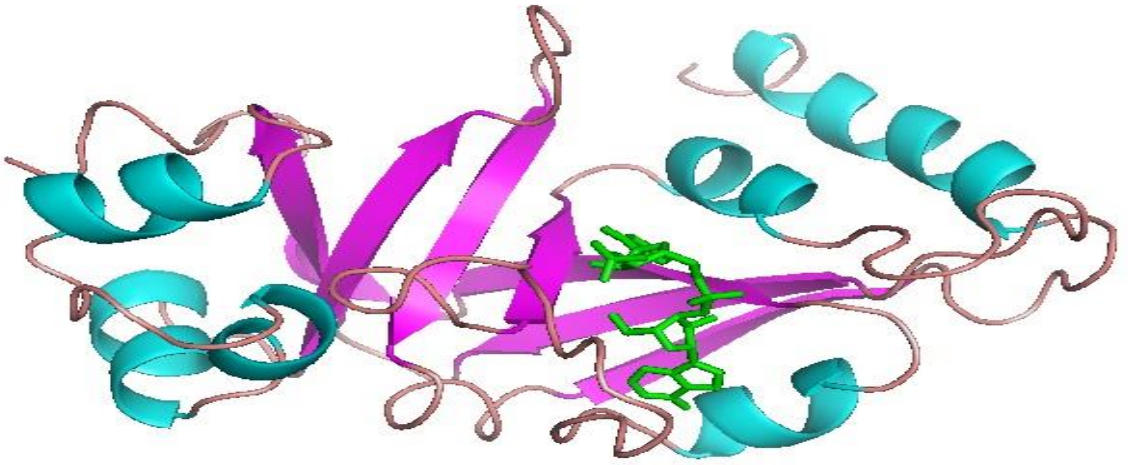

FIGURE 1.6 : RCSB downloaded structure of catalytic domain of diphtheria toxin (1DTP) with ligand ApUp attached.

1.9.4 New Delhi Metallo-β-Lactamase-1

1.9.4.1 Selection criteria

The discovery of New Delhi metallo- β-lactamase (NDM-1) for the first time was in a Swedish patient in a hospital in New Delhi [Yong et al. 2009]. After being discovered soon NDM-1 was reported in many other countries worldwide [Rolain et al. 2010]. The speed of the spread of NDM-1 positive strains is a cause of major concern [Nordmann et al. 2012]. The strains positive for NDM-1 are not inhibited by nearly all serine β-lactamase inhibitors commercially available.

1.9.4.2 Structure

The structure of NDM-1(FIGURE 1.7; pdb id: 3Q6X) has residues 65-73, which belong to the L1 active site loop and residues Ile35 and Lys216 were shown to be crucial for substrate binding and recognition [Zhu et al. 2013]. His120, His122 and His189 residues of NDM-1 are involved in coordination with Zinc1 (Zn1). Asp124, Cys208 and His250 residues of NDM-1 are involved in coordination with Zinc2 (Zn2). Lys211 and Asn220 residues of NDM-1 are involved in interaction with the β-lactams. Gln123 and Asp124 residues may contribute to substrate orientation and binding. Lys125 and Tyr229 contribute to the stability of Loop 6 (L6) and Loop 10 (L10) respectively [Zhang and Hao 2011].

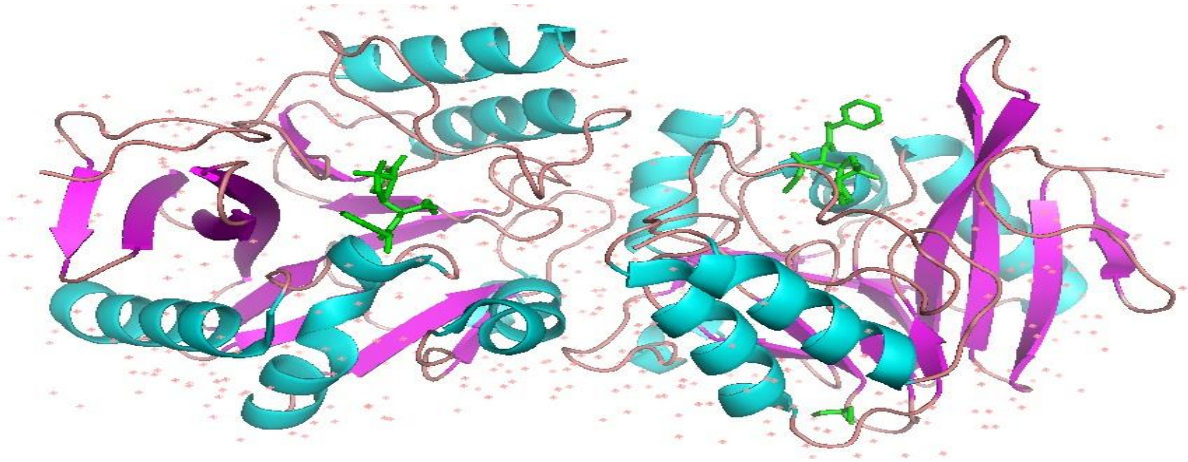

FIGURE 1.7: RCSB downloaded structure of New Delhi Metallo-β-lactamase with inhibitors attached.

1.10 OVERALL GOAL OF THE THESIS

The overall work in the thesis focuses on the interaction of various virulence proteins with short length antimicrobial peptides. The *In silico* interaction studies between virulence factors and antimicrobial peptides would be performed and analysed. Potential antimicrobial peptide should be chosen. The properties and applications of the chosen antimicrobial peptides should be studied. Further, the activity of the antimicrobial peptide should be checked.