MOLECULAR DOCKING STUDIES ON INTERACTIONS OF ANTIMICROBIAL PEPTIDES WITH BACTERIAL TARGETS

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BANGALORE

2023

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Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (Agriculture)

in

BIOINFORMATICS

By

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2023

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE, GKVK, BENGALURU

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CERTIFICATE

This is to certify that the thesis entitled **"Molecular docking studies on interactions of antimicrobial peptides with bacterial targets"** submitted in partial fulfilment of the requirement for the degree of **Master of Sciences (Agriculture)** in **Bioinformatics** to the University of Agricultural Sciences Bangalore is record of *bona fide* research work carried out by **Mr. CHARITH, R., PAMB 0218** during the period of his study in this University, under my guidance and supervision. The thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Place: Bengaluru

Month & Year: January, 2023

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3. **(SHASHIDHARA, K. S.)**

Affectionately Dedicated to

My Family and to the

Scary Corona

ACKNOWLEDGEMENT

Words were not enough to express my heartiest gratitude to those people whose assistance and care was indispensable for the completion of thesis.

First and foremost, I thank God for giving me the strength to undertake this research work and to persevere and complete it satisfactorily.

I would like to express my heartfelt respects and gratitude to the esteemed chairperson of my Advisory Committee ***Dr. Ramesh, B. N.****, Assistant Professor, ICAR-AICRP on, UAS Bengaluru for his invaluable guidance, co-operation, encouragement, and moral support throughout the period of my study. All of these finally shaped my present endeavor. I feel proud for the privilege of being his student.*

To the members of my advisory committee, I express my appreciation and deep gratitude to ***Dr. Veena S. Anil.,*** *Professor, Department of Plant Biotechnology, UAS Bengaluru,* ***Dr. Nagesha S. N.,*** *Assistant professor Dept. of Biotechnology CoA, Hassan,* ***Dr. Shashidhara K. S.,*** *Assistant Professor, Department of Genetics and Plant Breeding, CoA, Hassan, for their constant supervision, invaluable guidance extended during the course of this investigation. I am extremely grateful to my teachers* ***Dr. Shyamalamma, S****.,* ***Dr. K. M. Harinikumar****,* ***Dr. Anitha Peter, Dr. Veena, S. Anil, Dr. Nagesha, N., Dr. Benherlal, P., Mrs. Poornima, R., Dr. Nagesha, S. N., Dr. Prasanna, S. Koti, Dr. Megha, S., Dr. Pratheepa, Shodaka team*** *and non-teaching staff of the Department of Plant Biotechnology for their valuable suggestions, support and encouragement during my study period.*

I don't want to create a distance between us by saying a word thanks, I owe all my success to my beloved family members, mother ***Gayathri,*** *father* ***Rajashekara,*** *brother* ***Lishruth,*** *Sisters* ***Bhomika,*** *for their unconditional understanding and selfless true love. The right ways they taught, the sacrifices they made, the support they offered and their constant encouragement to see me for what I am today is priceless. I would also thank my mother side family members who supported me.*

I take pleasure to acknowledge the help, guidance and mutual cooperation rendered by my guidemates ***Nivi(Niveditha) and Hitha(Hithashree)*** *to complete my research.*

My most sincere thanks to my supporting classmates ***Nags(Nagraj), Rachna(Rachana), Vin(Vinod), Praj(Prajwal), Sowmya and Charitha(as CR)*** *Juniors* ***Smriti, Pushpa(Hariprasad), Vishwa, Akash, Vidhya(Vidhyadhar) and Shivu****. Favourite Senior* ***Suresh sir*** *and finally to my beautiful future* ***Peahen*** *being there all through this Masters journey and sharing beautiful unforgettable memories together.*

I believe that the word thanks will create a invisible distance so it's a great pleasure to not thank my unforgettable funfull friends ***Nags(Nagraj), Maha(Mahadesh), Kapoor(Shahid), Katthy(Sathya Seelan), Raaahul, Poojitha, (Nivi)Niveditha and Sahana*** *for being a special part of this Masters journey. I want this to happen like How a film will haunt even after coming out of theatre likewise our wonderful memories should (Hopefully).*

*A special special mention of my Lifelong friends '****BHARJARI BOYS****' Venu, Nitz, Sandy, Sharu, Pawan, Puli(Anthamma), Wild_Ganga, Manju_Yo, Nanda, Arvind_A P, Holur(Sanjay), Suman, Hemanth, Bhaagi(Bharath), Shashi anna and Harish Sir for their friendship, help, care, upliftment during my studies and worries. And also a mention of* ***'Akkimangla Boys' (Venu anna, Guru anna, Bharath anna, Palav and Sepayi), Subbi(Subhash),Messi(Rakesh), Phele(Rakesh), Niranjan and Madhu.***

I express my sincere gratitude to ***University of Agricultural Sciences,*** *Bangalore and* ***Department of Plant Biotechnology****(only its Area) for providing support for my Master's degree programme* ***which seeded many desires inside me and helped me to be as I want to****(close to ninety percentage).*

Bengaluru

January, 2023 (**Charith, R.**)

Molecular Docking studies on interactions of Antimicrobial peptides with Bacterial targets.

R. CHARITH, PAMB 0218

Department of Plant Biotechnology, UAS, GKVK, Bengaluru - 65

INTRODUCTION

- The rapid increase in drug-resistant infections has presented a serious challenge to antimicrobial therapies. The failure of the most potent antibiotics to kill "superbugs" emphasizes the urgent need to develop other control agents. The antimicrobial peptides (AMPs) are biologically active molecules produced by wide variety of organisms as an essential component of their innate immune response.
- AMPs are considered as a promising and potential drug candidate for the future due to their broad range of activity, lesser toxicity, and decreased resistance development by the target cells.
- To overcome drawback of traditional methods, more effective and rational methods have been introduced which rely on virtual screening. Based on the availability of structural information, the method of virtual screening can be classified as structure based and ligand-based drug designing method.
- The structure based drug designing approach describes molecular docking. Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand–protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization.

OBJECTIVES

❖ Screening of AMPs from Databases.

MATERIAL AND METHODS

APD3(Antimicrobial Peptide Database) :

- The antimicrobial peptide database is an original database initially online in 2003. The APD2 (2009 version) has been regularly updated and further expanded into the APD3.
- This database currently focuses on natural antimicrobial peptides (AMPs) with defined sequence and activity. It includes a total of 2619 AMPs with 261 bacteriocins from bacteria, 4 AMPs from archaea, 7 from protists, 13 from fungi, 321 from plants and 1972 animal host defense peptides.
- The APD3 contains 2169 antibacterial, 172 antiviral, 105 anti-HIV, 959 antifungal, 80 antiparasitic and 185 anticancer peptides. Newly annotated are AMPs with antibiofilm, antimalarial, anti-protist, insecticidal, spermicidal, chemotactic, wound healing, antioxidant and protease inhibiting properties.
- Identification of potential AMP's and screen of the AMP's based on the parameters which enhances the antimicrobial activity from the database.

AMP's based on peptide parameters like length, net charge, hydrophobic residues(%) and structure type like helix/beta/combined helix and beta forms.

Peptide Parameters	Description
Length	≤ 50
Net charge	5
Hydrophobic residues(%)	41-50
Structure type	Helix/Beta/Combined Helix and Beta

RESULTS & DISCUSSION

- With the AMP's parameters like Length, Net charge, Hydrophobic residues(%) and Structure type(Helix / Beta / Combined Helix and Beta).
- Identification of AMP's based on Parameters given below,
  1. Length, Net charge, Hydrophobic residues(%) and Helix : 19
  2. Length, Net charge, Hydrophobic residues(%) and Beta : 0
  3. Length, Net charge, Hydrophobic residues(%) and Combined Helix and Beta : 1
- Totally identified AMP's based on the parameters are 20

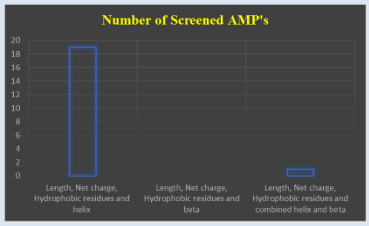
Number of Screened AMP's

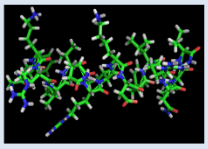
Parameters	Length, Net charge, Hydrophobic residues and helix	Length, Net charge, Hydrophobic residues and beta	Length, Net charge, Hydrophobic residues and combined helix and beta
Number of AMP's	19	0	1

SL.NO	AMP's	Sequence
1	AP00082	GIFSKLAGKKLKNLLISGLKNVGKEVGMDVVRTGIDIAGC KIKGEC
2	AP00135	GWLKKIGKKIERVGQHTRDATIQTIGVAQQAANVAATLK
3	AP00384	LLKELWTKIKGAGKAVLGKIKGLL
4	AP00608	KRIVQRIKDFLR
5	AP00708	GFKRIVQRIKDFLRNLV
6	AP01159	KWKIFKKIEHMGQNIRDGLIKAGPAVQVVGQAATIYKG
7	AP01259	RWKIFKKIEKVGQNIRDGIVKAGPAVAVVGQAATI
8	AP01656	GIFSKLAGKKIKNLLISGLKNIGKEVGMDVVRTGIDIAGCK IKGEC
9	AP01824	FLPKLFAKITKKNMAHIR
10	AP02612	KIKIPWGKVKDFLVGGMKAV
11	AP02318	IKIPSFFRNILKKVGKEAVSLIAGALKQS
12	AP02740	GLLSALRKMIPHILSHIKK
13	AP02872	GIFSKLAGKKIKNLLISGLKG
14	AP02900	TRWLWLLRGGLKAAGWGIRAHLNRNQ
15	AP02975	FFGHLFKLATKIIPSLFQRKKE
16	AP03166	GFSSLFKAGAKYLLKQVGKAGAQQLACKAANNC
17	AP03170	GFSSLFKAGAKYLLKQVGKAGAQQL
18	AP03200	FFGHLLRGIVSVGKHIHGLITG
19	AP03275	FLGSLFSIGSKLLPGVIKLFQRKKQ
20	AP00403	ACNFQSCWATCQAQHSIYFRRAFCDRSQCKCVFVRG

- Screening of 20 AMP's from the APD3 Database by considering some of the parameters which is essential for peptides to have antimicrobial activity.
- Selection of the bacterial targets of particular disease caused by bacterial pathogens and with the available AMP's, perform Molecular Docking to know the interaction between targets and AMP's.
- Based on the Binding affinity obtained from the results of Docking process, we consider the appropriate AMP's for the bacterial targets.

GRAPHS & PHOTOGRAPHS





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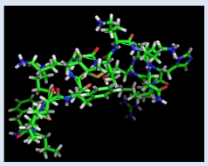
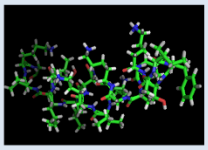


AP01824 (Andersonin-Y1)



AP02872 (Esculentin-1A)

SUMMARY

- AMPs are considered as a promising and potential drug candidate.
- From the APD3 database identification of AMP's based on essential parameters which enhances antimicrobial activity.
- Finally 20 AMP's are screened from the database.

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Dr. Nagesha, S.N.
Dr. Shashidhara, K.S.

MOLECULAR DOCKING STUDIES ON INTERACTIONS OF ANTIMICROBIAL PEPTIDES WITH BACTERIAL TARGETS

CHARITH, R.

THESIS ABSTRACT

Colibacillosis and Salmonellosis are the most common bacterial diseases in poultry industry. Many antibiotics have become susceptible to the bacterial pathogens like E coli and Salmonella. Finding new antibiotic alternatives is essential because antibiotic resistance brought on by misuse, particularly the growth of multi-drug resistant bacteria which poses a severe threat to human health. An efficient therapeutic approach must be developed immediately to control infections. AMPs have become powerful alternative agents that have met the need for novel anti-infectives to overcome increasing antibiotic resistance problems. The present work conducted protein-peptide docking by using Autodock Vina with the list of 10 screened AMPs against the poultry bacterial targets (PBP 6 and LptA proteins) and by using Discovery Studio which revealed different binding interaction with target proteins. The original unmodified AMP (AP00551) and the modified AMP (FFHLHFHYWWW) showed favourable interactions with highest binding affinity of -8.7 Kcal/mol and-8.6 Kcal/mol with penicillin binding protein of E coli. For Salmonella the original unmodified AMP (AP00551) and the modified AMP (FFHLHFHYWWW) showed favourable interactions with highest binding affinity of -9.4 Kcal/mol and -9.0 Kcal/mol with LptA protein. Molecular docking studies indicated that these peptides could be used to block PBP 6 which is responsible for cell morphology development of E coli and the LptA enzyme which is responsible for membrane synthesis of Salmonella. Based on the binding energy score, the 10 AMPs could serve as a therapeutic option against the bacterial targets and enhance its treatment outcome.

January, 2023

Department of Plant Biotechnology
UAS, GKVK, Bengaluru

Ramesh, B. N.
(Major advisor)

ಬ್ಯಾಕ್ಟೀರಿಯಾದ ಗುರಿಗಳೊಂದಿಗೆ ಆಂಟಿಮೈಕ್ರೊಬಿಯಲ್ ಪೆಪ್ಟೈಡ್‌ಗಳ ಪರಸ್ಪರ ಕ್ರಿಯೆಗಳ ಮೇಲೆ ಆಣ್ವಿಕ ಡಾಕಿಂಗ್ ಅಧ್ಯಯನಗಳು

ಚರಿತ್, ಆರ್

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಕೊಲಿಬಾಸಿಲೋಸಿಸ್ ಮತ್ತು ಸಾಲ್ಮೊನೆಲೋಸಿಸ್ ಕೋಳಿ ಉದ್ಯಮದಲ್ಲಿ ಸಾಮಾನ್ಯ ಬ್ಯಾಕ್ಟೀರಿಯಾದ ಕಾಯಿಲೆಗಳಾಗಿವೆ. ಅನೇಕ ಪ್ರತಿಜೀವಕಗಳು ಇಕೊಲಿ ಮತ್ತು ಸಾಲ್ಮೊನೆಲ್ಲಾನಂತಹ ಬ್ಯಾಕ್ಟೀರಿಯಾದ ರೋಗಕಾರಕಗಳಿಗೆ ಒಳಗಾಗುತ್ತವೆ. ಹೊಸ ಆಂಟಿಬಯೋಟಿಕ್ ಪರ್ಯಾಯಗಳನ್ನು ಕಂಡುಹಿಡಿಯುವುದು ಅತ್ಯಗತ್ಯ ಏಕೆಂದರೆ ದುರುಪಯೋಗದಿಂದ ಉಂಟಾಗುವ ಪ್ರತಿಜೀವಕ ನಿರೋಧಕತೆ, ವಿಶೇಷವಾಗಿ ಬಹು–ಔಷಧ ನಿರೋಧಕ ಬ್ಯಾಕ್ಟೀರಿಯಾದ ಬೆಳವಣಿಗೆಯು ಮಾನವನ ಆರೋಗ್ಯಕ್ಕೆ ತೀವ್ರ ಅಪಾಯವನ್ನುಂಟು ಮಾಡುತ್ತದೆ. ಸೋಂಕುಗಳನ್ನು ನಿಯಂತ್ರಿಸಲು ಸಮರ್ಥ ಚಿಕಿತ್ಸಕ ವಿಧಾನವನ್ನು ತಕ್ಷಣವೇ ಅಭಿವೃದ್ಧಿಪಡಿಸಬೇಕು. AMP ಗಳು ಪ್ರಬಲ ಪರ್ಯಾಯ ಏಜೆಂಟ್‌ಗಳಾಗಿ ಮಾರ್ಪಟ್ಟಿವೆ, ಅವುಗಳು ಹೆಚ್ಚುತ್ತಿರುವ ಪ್ರತಿಜೀವಕ ನಿರೋಧಕ ಸಮಸ್ಯೆಗಳನ್ನು ನಿವಾರಿಸಲು ನವೀನ ವಿರೋಧಿ ಸೋಂಕುಗಳ ಅಗತ್ಯವನ್ನು ಪೂರೈಸಿವೆ. ಪೌಲ್ಟ್ರಿ ಬ್ಯಾಕ್ಟೀರಿಯಾ ಗುರಿಗಳ (PBP 6 ಮತ್ತು Lpt A ಪ್ರೋಟೀನ್‌ಗಳು) ವಿರುದ್ಧ ಪರೀಕ್ಷಿಸಲಾದ ೧೦ AMP ಗಳ ಪಟ್ಟಿಯೊಂದಿಗೆ ಆಟೋಡಾಕ್ ವಿನಾವನ್ನು ಬಳಸುವ ಮೂಲಕ ಪ್ರೋಟೀನ್–ಪೆಪ್ಟೈಡ್ ಡಾಕಿಂಗ್ ಅನ್ನು ನಡೆಸಿತು ಮತ್ತು ಡಿಸ್ಕವರಿ ಸ್ಟುಡಿಯೊವನ್ನು ಬಳಸುವ ಮೂಲಕ ಗುರಿ ಪ್ರೋಟೀನ್–ಪೆಪ್ಟೈಡ್‌ಗಳ ವಿಭಿನ್ನ ಬೈಂಡಿಂಗ್ ಪರಸ್ಪರ ಕ್ರಿಯೆಯನ್ನು ಬಹಿರಂಗಪಡಿಸಿತು. ಮೂಲ ಮಾರ್ಪಡಿಸದ AMP (AP00551) ಮತ್ತು ಮಾರ್ಪಡಿಸಿದ AMP (FFHLHFHYWWW) ಬೈಂಡಿಂಗ್ ಎನರ್ಜಿ –೮.೭ Kcal/mol ಮತ್ತು –೮.೬ Kcal/mol ನ ಪೆನ್ಸಿಲಿನ್ ಬೈಂಡಿಂಗ್ ಪ್ರೋಟೀನ್‌ನೊಂದಿಗೆ ಇಕೊಲಿನ ಅತ್ಯಧಿಕ ಬಂಧಿಸುವ ಸಂಬಂಧದೊಂದಿಗೆ ಅನುಕೂಲಕರವಾದ ಪರಸ್ಪರ ಕ್ರಿಯೆಗಳನ್ನು ತೋರಿಸಿದೆ. ಸಾಲ್ಮೊನೆಲ್ಲಾಗೆ ಮೂಲ ಮಾರ್ಪಡಿಸದ AMP (AP00551) ಮತ್ತು ಮಾರ್ಪಡಿಸಿದ AMP (FFHLHFHYWWW) –೯.೪ Kcal/mol ಮತ್ತು –೯.೦ Kcal/mol ಪ್ರೋಟೀನ್‌ನ ಅತ್ಯಧಿಕ ಬಂಧಕ ಸಂಬಂಧದೊಂದಿಗೆ ಅನುಕೂಲಕರವಾದ ಪರಸ್ಪರ ಕ್ರಿಯೆಗಳನ್ನು ತೋರಿಸಿದೆ. ಆಣ್ವಿಕ ಡಾಕಿಂಗ್ ಅಧ್ಯಯನಗಳು ಈ ಪೆಪ್ಟೈಡ್‌ಗಳನ್ನು PBP 6 ಅನ್ನು ನಿರ್ಬಂಧಿಸಲು ಬಳಸಬಹುದೆಂದು ಸೂಚಿಸಿದೆ, ಇದು ಇಕೊಲಿಯ ಜೀವಕೋಶದ ರೂಪವಿಜ್ಞಾನದ ಬೆಳವಣಿಗೆಗೆ ಕಾರಣವಾಗಿದೆ ಮತ್ತು ಸಾಲ್ಮೊನೆಲ್ಲಾದ ಪೊರೆಯ ಸಂಶ್ಲೇಷಣೆಗೆ ಕಾರಣವಾದ Lpt A ಕಿಣ್ವವಾಗಿದೆ. ಬೈಂಡಿಂಗ್ ಎನರ್ಜಿ ಸ್ಕೋರ್ ಅನ್ನು ಆಧರಿಸಿ, ೧೦ AMP ಗಳು ಬ್ಯಾಕ್ಟೀರಿಯಾದ ಗುರಿಗಳ ವಿರುದ್ಧ ಚಿಕಿತ್ಸಕ ಆಯ್ಕೆಯಾಗಿ ಕಾರ್ಯನಿರ್ವಹಿಸುತ್ತವೆ ಮತ್ತು ಅದರ ಚಿಕಿತ್ಸೆಯ ಫಲಿತಾಂಶವನ್ನು ಹೆಚ್ಚಿಸಬಹುದು.

ಜನವರಿ, ೨೦೨೩.

ಸಸ್ಯ ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ — **ರಮೇಶ್, ಬಿ. ಎನ್.**
ಕೃಷಿ ಮಹಾವಿದ್ಯಾನಿಲಯ, ಗಾ. ಕೃ. ವಿ. ಕೇ., ಬೆಂಗಳೂರು–೫೬೦೦೬೫ — (ಮುಖ್ಯ ಸಲಹಾಗಾರರು)

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LIST OF ABBREVIATIONS

Sl. No.	Abbreviations	Expand form
1.	AMPs	Antimicrobial peptide
2.	*et al.,*	Coworkers
3.	APD	Antimicrobial peptide database
4.	A^0	Angstrom
5.	ADT	AutoDock Tools
6.	PDB	Protein data bank
7.	PBP	Penicillin binding protein
8.	Da	Dalton
9.	Kcal	Kilo calorie
10.	SVM	Support Vector Machine
11.	VS	Virtual screening
12.	ALA	Alanine
13.	VAL	Valine
14.	PHE	phenylalanine
15.	PRO	Proline
16.	SER	Serine
17.	ARG	Arginine
18.	THR	Threonine
19.	GLY	Glycine
20.	HIS	Histidine
21.	TRP	Tryptophan
22.	UCSF	University of California, San Francisco
23.	SVM	Support Vector Machine
24.	CADD	Computer-Aided Drug Design

INTRODUCTION

I INTRODUCTION

One of the most common food sectors worldwide is poultry. Chicken is the most commonly farmed species and a large diversity of antimicrobials are used to raise poultry in most countries. Consuming meat and poultry has been linked to many foodborne illnesses. The digestive health of chickens is at risk from enteric bacterial infections, which can also affect a flock's liveability and feed efficiency. Salmonellosis and colibacillosis are two common enteric bacterial infections that affect different organ systems than the intestine in chicken (Porter, 1998).

Any localised or systemic infection caused entirely or in part by avian pathogenic *Escherichia coli* (APEC) is referred to as colibacillosis. This includes colisepticemia, hemorrhagic septicemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease), swollen-head syndrome, venereal colibacillosis. *Escherichia coli* is a typical bacterium that naturally exists in the environment and in the gastrointestinal system of birds. However, it can result in a clinical scenario known as colibacillosis, among the most widespread infections in poultry. Colibacillosis is often one of the most frequently reported infections, according to the poultry health surveys (Nolan *et al*., 2013).

Solmonella, a type of bacteria, is what causes salmonellosis in chickens. The pathogen is Gram negative bacteria, non-spore-forming, and motile *Bacillus* belonging to the Enterobacteriaceae family. It is a facultative anaerobe that grows at pH levels between 4 and 8 and temperatures between 8^0 and 45^0 Celsius. Based on host adaptation and infectiousness, the pathogen is widely characterised as typhoidal. Additionally, zoonotic relevance is associated with motile Salmonellae (paratyphoid group) infection, which causes salmonellosis in chickens (Feasey *et al*., 2012). Avian *Salmonella* infections are significant as a source of food-borne disease transmission to people as well as a cause of clinical disease in chickens. Only around 10% of more than 2,300 *Salmonella* serotypes that have been identified have been isolated from chicken. The naturally occurring hosts for the highly host-adapted biovars *S. pullorum* and *S. gallinarum* are chickens (Shivaprasad, 2000).

Microbial infections contribute substantially to global mortality trends. Although antibiotics were once capable of treating most bacterial infections, the emergence of antimicrobial resistance diminished the effectiveness of existing antibiotics. The design of antimicrobials that are less susceptible to evolutionary resistance mechanisms than conventional antibiotics is challenging. Bacteria possess three defined types of antimicrobial resistance: intrinsic, acquired and phenotypic or adaptive resistance. Despite their known capability to elicit bacterial resistance mechanisms, small-molecule antimicrobials remain at the forefront of initiatives to replace antibiotics that are beginning to fail. Antimicrobial peptides (AMPs), a diverse group of bioactive small proteins, are part of the body's first line of defense for pathogen inactivation. They work by disrupting bacterial cell membranes, modulating the immune response, and regulating inflammation. AMP reservoirs and expansion beyond the available chemical space remains high because there is a plethora of unconventional sources of AMPs, including unculturable soil and marine bacteria, and methods available to produce vast libraries of derivatives (Trimble *et al*., 2016).

The modern drug development process is heavily reliant on computational techniques, such as lead optimization techniques and virtual screening (VS) procedures for hit detection. A more rational and direct approach to drug discovery than conventional experimental high throughput screening. VS has the advantage of cheap cost and efficient screening (Moitessier *et al*., 2008). The two types of virtual screening are ligand-based and structure-based techniques. Since the early 1980s, molecular docking has been the most popular technique in structure-based drug creation (Kuntz *et al*., 1982).

Computational docking methods have proven to be helpful in the discovery and design of small-molecule drugs. Peptide therapeutics is a field where related attempts are being made. However, the docking methods designed for small molecule interactions are typically not a good fit for modeling of the significantly more flexible and larger peptide molecules. The interest in peptide therapeutics triggered the rapid development of new techniques dedicated to protein-peptide docking, which are getting more and more integrated into the process of developing new drugs. The applicability of AutoDock Vina is limited to short peptides of few amino acids (Ciemny *et al*., 2018).

We can characterise how Antimicrobial peptides behave at the binding site of target proteins and better understanding of fundamental biological processes by an atomic-level

interaction between a peptide and a protein using Molecular Docking method and it involves two fundamental key steps: evaluation of the binding affinity, prediction of the ligand (Peptide) structure, as well as its placement and orientation within these sites (commonly referred to as pose) (McConkey *et al*., 2002).

Our goal in this project is to identify novel antimicrobial peptides along with appropriate scientific reasoning, that is effective against bacterial infections such as colibacillosis and salmonellosis in the poultry, especially those caused by multidrug-resistant pathogens with the research entitled "Molecular docking studies on interactions of antimicrobial peptides with bacterial targets" with the following objectives:

1. Screening of AMPs from database.
2. Molecular docking of screened AMPs against bacterial target in poultry diseases.
3. *In silico* evaluation by molecular docking of modified AMPs against bacterial target in poultry diseases.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

2.1 Poultry Diseases

Diseases of Poultry is the most comprehensive reference for all aspects of poultry health and diseases, including pathogenesis, diagnostics, epidemiology, and control methods. Enteric bacterial infections in poultry pose a threat to intestinal health and can contribute to poor feed efficiency and livability of a flock. A variety of enteric bacterial diseases are recognized in poultry. Three of these bacterial diseases, necrotic enteritis, ulcerative enteritis, and spirochetosis, primarily infect the intestine, whereas other bacterial diseases, such as salmonellosis, colibacillosis, mycobacteriosis, erysipelas, and fowl cholera, affect a variety of organ systems in addition to the intestine (Porter, 1998).

Studies on the prevalence of gastrointestinal parasites of chicken reared under backyard and intensive systems were carried out in two north Indian states viz., Uttar Pradesh and Uttarakhand. Out of 58 poultry farms screened for gastrointestinal parasites, 81.03 % were positive for *Eimeria* spp., 15.52 % for *Ascaridia galli*, 3.45 % for *Hetarakis gallinarum*, 1.72 % for *Syngamus trachea*, 5.17 % for *Capillaria* spp, 1.72 % for *Raillietina* spp., 1.72 % for *Trichostrongylus tenuis*, 1.72 % for *Choanotaenia infundibulum* and 1.72 % for *Strongyloides avium*. The prevalence of helminthic infections was higher in poultry farms of Uttarakhand (40.0 %) as compared to Uttar Pradesh (11.62 %) with higher prevalence in backyard poultry (36.4 %), followed by layer farms (28.6 %) and lowest in broiler farms (9.1 %) (Kumar *et al*., 2015).

The Andaman and Nicobar islands witnessed a tragic incidence of major earthquake followed by tsunami in the year 2004 due to which the loss of livestock was recorded as poultry (19.35%), cattle (10.3%), goat (37.75%) and pig (83.55%). However, the incidence and prevalence of parasitic diseases are very high due to the high humidity and rainfall which favour the prevalence of parasitic diseases. The post-tsunami disease trends of the livestock and poultry indicated the outbreak of some of the diseases which were never reported from the Islands earlier. The diseases were introduced probably due to the entry of livestock, meat, meat products, egg, and poultry from mainland (Sundar, 2014).

2.1.1 Bacterial diseases in Poultry

Many foodborne diseases are associated with consumption of meat and poultry. Some pathogens were not previously known (new pathogens), others have newly arisen as foodborne (emerging pathogens), and others have become more potent or associated with other products (evolving pathogens). Many of these pathogens may cause severe illness, besides gastroenteritis. *Campylobacter jejuni* is a leading cause of food-associated bacterial illness; *Campylobacter jejuni* O:19 and other serotypes are common etiological agents of Guillain–Barré syndrome, a neuropathy due to autoimmune response. *Salmonella typhimurium* DT104 and other serotypes have been found to be multi-drug resistant; salmonellosis may lead to chronic reactive arthritis. Many outbreaks of enterohemorrhagic *Escherichia coli* have been associated with consumption of undercooked contaminated ground beef (Mor-Mur and Yuste, 2010).

Commercial poultry farms frequently use live bacterial prophylactics like vaccines and probiotics to prevent bacterial infections. Due to the emergence of antibiotic-resistant bacteria in poultry animals, a closer examination into the health benefits and limitations of commercial, live prophylactics as an alternative to antibiotics is urgently needed. They summarize the peer-reviewed literature of several commercial live bacterial vaccines and probiotics. As per their estimation, there is a paucity of peer-reviewed published research regarding these products, making repeatability, product-comparison, and understanding biological mechanisms difficult. Increased emphasis on these areas would open several avenues for research, ranging from improving protection against bacterial pathogens to using these prophylactics to modulate animal behavior (Redweik, 2020).

2.1.1.1 Colibacillosis in poultry

Avian colibacillosis is caused by a group of pathogens designated avian pathogenic *Escherichia coli* (APEC). Despite being known for over a century, avian colibacillosis remains one of the major endemic diseases afflicting the poultry industry worldwide. Autologous bacterins provide limited serotype-specific protection, yet multiple serogroups are associated with disease, especially O1, O2 and O78 among many others (Yuvraj, 2019).

Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia coli* (APEC), including colisepticemia, hemorrhagic septicemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), swollen-head syndrome, venereal colibacillosis, coliform cellulitis (inflammatory or infectious process, IP), peritonitis, salpingitis, orchitis, osteomyelitis/ synovitis (including turkey osteomyelitis complex), panophthalmitis, omphalitis/yolk sac infection, and enteritis. Often colibacillosis is among the most frequently reported diseases in surveys of poultry health or condemnations at processing (Nolan *et al*., 2013).

2.1.1.1.1 Etiology

The disease is caused by certain strains of avian pathogenic *Escherichia coli* (APEC), of which strain O is more common. *Escherichia coli* is a gram-negative, non-acid fast, non-staining, non-spore-forming *Bacillus*, usually 3 × 0.6 μm in size, which is included in the Enterobacteriaceae family (Nolan *et al*., 2013).

E. coli is usually motile, often fimbriated, with peritrichous flagella. Somatic (O), flagellar (H) and sometimes capsular (K) antigens are used to differentiate *E. coli* serotypes. The pathogenic strains of the bacteria have virulence factors that allow them to colonize mucosal surfaces and subsequently produce diseases. Age, immune status, the nature of the diet and the strong exposure to pathogenic strains are the factors that predispose animals to develop clinical diseases after colonization by the pathogen. (Quinn *et al*., 2011).

2.1.1.1.2 Epidemiology

The organism is present throughout the world and is a normal inhabitant of the gastrointestinal tract of animals and birds at a concentration of 106 per grams, while the dust in the poultry house can contain up to 105 -106 / gm. Garbage and feces are sources of contamination for both healthy birds and eggs. Transmission is possible through ovaries to newborn chicks. Food, rodent droppings, contaminated well water are the sources of pathogenic *E. coli*. While, isolates of pathogens for poultry commonly belong to certain

serogroups, particularly serogroups O78, O1 and O2, but O15 and O55 may also be found to some extent (Chart *et al*., 2000).

The most frequent serogroup of *E. coli* observed was O76 (15.59%), followed by 14.45% O8, 12.17% O1, 7.22% O26, 6.44% O2, 4.94% O114, 4.18% O11, 3.80 % O2 and 3.04% every O45 and O84. Avian colibacillosis was found to be widely prevalent in all chicken age groups (9.52 to 36.73%) with an especially high prevalence rate in adult layer birds (36.73%) in Gazipur,

Bangladesh. However, the prevalence was found to be higher in broilers than in layers in the large Mymensingh district of Bangladesh and in the state of Kassala, East Sudan (Omer *et al*., 2010). The onset of colibacillosis was 32.52% during March 23, 2014 to May 14, 2014 in Gazipur, Bangladesh (Hossain *et al*., 2015).

2.1.1.1.3 Symptomatology

In general, broilers approximately 5 weeks of age are affected. Birds of any age can be affected if resistance is reduced by predisposing factors. Chicks less than 10 days old are particularly susceptible if their resistance has been reduced. The severity of the infection may be mild or persistent, and clinical signs may be absent in these cases. Birds with colisepticemia can become lethargic and stop eating and drinking. Severity of the disease may be indicated by the degree of reduced water consumption (Nolan *et al*., 2013).

2.1.1.1.4 Diagnosis

The diagnosis is based on the isolation and identification of the *E. coli* organism of the typical colibacillosis lesions. Fibrinous pericarditis is highly suggestive of the disease (Seneviratna, 1969). A presumptive diagnosis of *E. coli* infection can be made if the majority of the colonies are characteristically dark with a metallic glow on eosin-methylene blue (EMB) agar, bright pink, with a precipitate around the colonies on MacConkey agar, or yellow in tergitol-7 agar. Differentiation between APEC and commensal *E. coli* isolates can be made by using molecular diagnoses such as PCR. Acute septic diseases caused by *Pasteurellae (Pasteurella, Ornithobacterium, riemerella*), *Salmonella*e, *Streptococci* and other organisms, should be taken in consideration to differentiate the disease.

Chlamydophila, *pasteurellae* or *streptococci* (*Streptococcus, Enterococcus*) can cause pericarditis or peritonitis, and other bacteria, mycoplasmas and Chlamydophila can cause air saculitis (Nolan *et al*., 2013).

2.1.1.1.5 Treatment

Treatment strategies should include actions to control predisposing infections or environmental factors and the early use of antibacterials indicated by susceptibility testing. Different antimicrobial drugs have been used to treat and control colibacillosis. The fortuitous use of antimicrobials has produced a threat of resistance to antimicrobials and, therefore, the use of availability of effective antimicrobials has decreased. In addition, the development of new drugs is also lacking. Neomycin (38.00%) was mainly used for the treatment of colibacillosis, with an efficacy of 92.10%, with resistance of *E. coli* to neomycin, oxytetracycline, amoxicillin, enrofloxacin and ciprofloxacin, 38.88%, 50.55%, 60.68%, 50.00% and 30.55% respectively, during March 23, 2014 to May 14, 2014 in Gazipur, Bangladesh (Hossain *et al*., 2015). Gentamicin (100%) was highly sensitive for the treatment of colibacillosis (Hossain *et al*., 2015).

2.1.1.1.6 Receptor

Avian colibacillosis caused by the zoonotic pathogen *Escherichia coli* is a common bacterial infection that causes major losses in the poultry sector. Extracts of different medicinal plants and antibiotics have been used against poultry bacterial pathogens. However, overuse of antibiotics and extracts against pathogenic strains leads to the proliferation of multi-drug resistant bacteria. Due to their environmentally friendly nature, nanotechnology and beneficial bacterial strains can be used as effective strategies against poultry infections. Green synthesis of zinc oxide nanoparticles (ZnO-NPs) from Eucalyptus globulus leaves was carried out in this study. The maximum relative inhibition shown by ZnO-NPs, PGPR strains and eucalyptus leaf extracts was 88%, 67% and 58%, respectively. The effectiveness of ZnO-NPs was also increased with an increase in particle dose and treatment timeHowever, combined application of PGPR (Pseudomonas sp. (HY8N)) and ZnO-NPs augment antagonistic effects and showed maximum 69% antagonism. The study intends to investigate the binding affinity of ZnO-NPs with the

suitable receptor of the bacterial pathogen by *in silico* methods. The binding site conformations showed that the ligand ZnO binds with conserved binding site of penicillin-binding protein 6 (PBP 6) receptor. According to the interactions, ZnO-NPs form the same interaction pattern with respect to other reported ligands, hence it can play a significant role in the inhibition of PBP 6 (Masood *et al*., 2021).

The bacterial cell wall, also referred to as the cross-linked peptidoglycan, is constructed of a repeating disaccharide unit of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), with NAM bearing a peptide moiety. Cell wall polymerization includes two enzymatic reactions, a transglycosylation that links the disaccharides to form the glycan backbone and a transpeptidation that cross-links the peptides, creating a mesh-like structure. The synthesis and remodeling of bacterial cell wall is performed mainly by penicillin-binding proteins (PBPs), which are classified into high-molecular mass (HMM) and low-molecular mass (LMM) subgroups. These PBPs are the primary targets of the beta-lactam antibiotics. DD-Carboxypeptidases are members of LMM PBPs that cleave the peptide bond between the two terminal D-alanines of the muramyl peptide, an alteration in the peptidoglycan structure that moderates the degree of cross-linking of the cell wall. There are two primary DD-carboxypeptidases in *E coli*, PBP5 and PBP6. There have been few studies on PBP6 and the biological roles of these DD-carboxypeptidases remain uncertain. DD-Carboxypeptidases are not critical for bacterial growth in cultured media, but their functions are necessary for the health of the organism, especially for maintaining normal cell morphology. They investigate by X-ray crystallography both the specifics of PBP6 structure and function, and general questions of substrate and inhibitor recognition in this family of enzymes. They present the first X-ray structures of PBP6 from *E. coli*, whose apo enzyme structure we determine at 2.1 A^0. The synthesis of a key peptidoglycan fragment containing the N-acetyl-muramyl (NAM) moiety bearing the complete pentapeptide unit (L-Ala-D-isoGlu-L-Lys-D-Ala-D-Ala), allowed them to explore a complex between the wild-type PBP6 and a full muramyl peptide substrate, for the first time. They determined the X-ray structure of the pre-acylation complex ("Michaelis complex") with this substrate fragment and an acyl-enzyme complex with the antibiotic ampicillin to 1.8 A^0. Together with earlier DD-carboxypeptidase structures, these three

structures enable a detailed structural analysis of the reaction coordinates of these LMM PBPs, and speak to longstanding questions of the correspondence between beta-lactams and the peptidoglycan substrates they are thought to mimic. The apo-PBP6 active site resembles that of PBP5. Most active site residues adopt the same conformations among all four monomers, including the Lys209 Thr210 Leu128, Ile189, Arg190, Arg194, Lys209, Ile243, Arg244, Phe245 from the Lys-Thr-Gly triad (Chen *et al*., 2009).

2.1.1.2 Salmonellosis

The incidence of *Salmonella* in 60 samples of poultry products of national origin available for consumers obtained from two local butcher shops and one canteen of the city of Porto and the susceptibility to antimicrobial agents allowed for human or animal therapy were evaluated. The results show that poultry samples are frequently contaminated with *Salmonella* (60%), belonging to 10 different serotypes. *Salmonella enteritidis* and *S. hadar* were the most prevalent serotypes. In addition, a high number (75%) of the *Salmonella* isolates was resistant to one or more antimicrobial agents and eight different resistance profiles were recorded. Resistance to nalidixic acid and enrofloxacin was demonstrated for 50% of the isolates and the occurrence of resistant and multi resistant *S. enteritidis* isolates were less frequent than for *S. hadar*. This study suggests a high incidence of *Salmonella* on Portuguese poultry products and shows that they could be a potential vehicle of resistant *Salmonella* foodborne infections (Antunes *et al*., 2003).

2.1.1.2.1 Etiology

Solmonellosis in chicken is caused by a species of bacteria known as *Solmonella*. The pathogen is Gram negative bacteria, non-spore-forming, and motile *Bacillus* belonging to the Enterobacteriaceae family. It is a facultative anaerobe that develops at temperatures ranging from 8 to 45 degrees Celsius and at pH levels ranging from 4 to 8. Based on host adaptation and infectiousness, the pathogen is widely characterised as typhoidal or NTS. Avian *Salmonella* infection may occur in poultry either acute or chronic form by one or more member of genus *Salmonella*, under the family Enterobacteriaceae. Besides, motile *Salmonella*e (paratyphoid group) infection cause salmonellosis in chickens and have zoonotic significance. (Feasey *et al*., 2012)

2.1.1.2.2 Epidemiology

Avian *Salmonella* infections are important as both a cause of clinical disease in poultry and as a source of food-borne transmission of disease to humans. Under the family of Enterobacteriaceae, the genus *Salmonella* is a facultative intracellular pathogen causing localized or systemic infections; as well as a chronic asymptomatic carrier state. The etiological agent of fowl typhoid and pullorum disease is *Salmonella* enterica sub sp. *enterica* serovar Gallinarum, which is divided into two distinct biovars under the serogroup D1, Gallinarum and Pullorum, which are denoted as *S. gallinarum* and *S. pullorum*, respectively (Shivaprashad, 1997).

In addition to *S. gallinarum* and *S.pullorum*, other *Salmonella*e such as *S. enteritidis*, *S. panama* and *S. dublin* also belong to the serogroup D1. The various motile and non-host adapted highly invasive serotypes such as *Salmonella enteritidis* and *Salmonella typhimurium* are commonly referred to as paratyphoid *Salmonella*e. Age wise prevalence of avian salmonellosis showed highest infection rate in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens (Gast, 1997).

Although more than 2,300 serotypes of *Salmonella* have been identified, only about 10% of these have been isolated from poultry. Chickens are the natural hosts for the highly host adapted biovar *S. gallinarum* and *S. pullorum*, but natural outbreaks have also been reported in turkeys, guinea fowl, quail and pheasants. Fowl typhoid is a per acute, acute or chronic form of disease affecting mostly adult chickens, whereas pullorum disease affects the very young chickens, mostly 2−3 weeks of age. In the adult it tends to be chronic. Fowl typhoid is frequently referred to as a disease of adult birds; still, there are also reports of high morbidity and mortality in young chickens. *S. gallinarum* can produce lesions in chicks, which are indistinguishable from those associated with pullorum disease. A certain percentage of chickens that survive from the initial infection become carriers with or without presence of clinical signs and pathological lesions (Shivaprasad, 2000).

2.1.1.2.3 Symptomatology

The main site of multiplication of these bacteria is the digestive tract, which may result in widespread contamination of the environment due to bacterial excretion through feces. Following invasion through the intestinal mucosa, cecal tonsils and Peyer's patches, the organisms are engulfed by macrophages, and through the blood stream and/or lymphatic systems, they spread to organs rich in reticuloendothelial tissues (RES), such as liver and spleen, which are the main sites of multiplication. In case of inadequate body defense mechanism, they may lead to second invasion and be localized in other organs, particularly ovary, oviduct, myocardium, pericardium, gizzard, yolk sac and/or lungs (Barrow, 1997).

In the bird challenge, *S. typhimurium* rapidly caused inflammation of the intestinal mucosa, but *S. pullorum* preferentially targeted the bursa of fabricius prior to eliciting intestinal inflammation. Pullorum disease manifests itself predominantly as an enteric disease of chickens, while fowl typhoid shows signs of septicemic disease. Both biovars can cause septicemic infections, which may be acute or chronic, but unlike *S. pullorum*, *S. gallinarum* is capable of producing peracute infection and hemolytic anemia in both young and adults. *S. gallinarum* is extremely pathogenic to young broiler chicks (Lowry *et al*., 1999).

2.1.1.2.4 Diagnosis

Diagnosis of avian salmonellosis should be confirmed by isolation, identification, and serotyping of *Salmonella* strains. Infections in mature birds can be identified by serologic tests, followed by necropsy evaluation complemented by microbiologic culture and typing for confirmation. A serological ELISA test for the diagnosis of avian salmonellosis either with *S. typhimurium or S. enteritidis* has been established. A diagnostic and a real-time PCR system for rapid and reliable genus- and serovar- (*S. enteritidis and S. typhimurium*) specific detection of *Salmonella* for monitoring purposes in the poultry food chain (Szmolka *et al*., 2006).

2.1.1.2.5 Treatment

Although fowl typhoid and pullorum disease are widely distributed in most parts of the world, the diseases have been eradicated from commercial poultry in developed countries such as the United States of America, Canada and most countries of Western Europe. Successful control programs can be achieved by developing good hygiene and management together with routine serological tests and slaughter policy. The principal management procedures should include chicks free from infections, and the chicks should be placed in a cleaned, sanitized and *S. gallinarum and S. pullorum* free environment with strict biosecurity measures. The feed and water should be free from *Salmonella* contamination. The dead birds need to be well disposed. Adequate precautions are needed to prevent infections from mechanical carriers like footwear, human clothing, hatchery disciplines, equipments, litters, crates, trucks and processing plants (Christensen *et al*., 1994).

The birds need to be tested at the age of 16 weeks due to immunologic maturity, at the point of lay due to stress and two consecutive times one month apart to provide the acceptable evidence that the flock is free from fowl typhoid. The potential role of probiotics for the controlling of *Salmonella* strains of poultry via the mechanisms of competitive exclusion. Vaccines may be used to control the disease, and antibiotics can be used for the treatment of fowl typhoid and pullorum disease (Lutful, 2009).

2.1.1.2.6 Receptor

Colistin (polymyxin E) is widely used in animal and human medicine and is increasingly used as one of the last-resort antibiotics against Gram-negative *Bacilli*. Due to the increased use of colistin in treating infections caused by multidrug-resistant Gram-negative bacteria, resistance to this antibiotic ought to be monitored. The study was undertaken to elucidate the molecular mechanisms, genetic relationships and phenotype correlations of colistin-resistant isolates. Here, They reported the detection of the mcr-1 gene in chicken-associated *Salmonella* isolates in Bangladesh and its *in silico* functional analysis. Out of 100 samples, 82 *Salmonella* spp. were isolated from chicken specimens (liver, intestine). When screened using PCR, five out of ten *Salmonella* isolates were found

to carry the mcr-1 gene. One isolate was confirmed for *Salmonella enterica* subsp. enterica serovar Enteritidis, and other four isolates were confirmed for *Salmonella* enterica subsp. *enterica* serovar *Typhimurium*. Sequencing and phylogenetic analysis revealed a divergent evolutionary relationship between the catalytic domain of *Neisseria meningitidis* lipooligosaccharide phosphoethanolamine transferase A (LptA) and MCR proteins, rendering them resistant to colistin. Three-dimensional homology structural analysis of MCR-1 proteins and molecular docking interactions suggested that MCR-1 and LptA share a similar substrate binding cavity, which could be validated for the functional analysis. The comprehensive molecular and *in silico* analyses of the *E coli* colistin resistance mcr-1 gene of *Salmonella spp*. of chicken origin in the present study highlighted the importance of continued monitoring and surveillance for antimicrobial resistance among pathogens in food chain animals (Uddin *et al*., 2022).

High mortality rate associated with multidrug-resistant (MDR) gram-negative bacterial infections. Increasingly, colistin is used for the treatment of sepsis caused by carbapenemase-producing gram negative bacteria. However, this treatment has resulted in the selection of MDR bacterial isolates with chromosomal resistance mutations in lipid A biosynthesis and regulation and more recently, the appearance of a mobile colistin resistance gene (mcr-1). Catalytic domain of *Neisseria meningitidis* lipooligosaccharide phosphoethanolamine transferase A (LptA) and MCR proteins, rendering them resistant to colistin. Three-dimensional homology structural analysis of MCR-1 proteins and molecular docking interactions suggested that MCR-1 and LptA share a similar substrate binding cavity, which could be validated for the functional analysis. Mcr-1 encodes a member of the family of phosphoethanolamine (PEA) transferases that decorates the lipid A headgroups of lipopolysaccharide with PEA. The 3D structure of LptA (PDB ID: 5FGN; *Neisseria meningitidis*), the best template of MCR-1 of *Salmonella* SAUVM isolates. Most active site residues are Lys142, Lys144, Arg146, Lys150 His453, Asp452, Glu240, and Thr280 (Anandan *et al*., 2017).

2.2 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are effector molecules of the innate immune system. A variety of AMPs have been isolated from species of all kingdoms and are classified based on their structure and amino acid motifs. AMPs have a broad antimicrobial spectrum and lyse microbial cells by interaction with biomembranes. Besides their direct antimicrobial function, they have multiple roles as mediators of inflammation with impact on epithelial and inflammatory cells influencing diverse processes such as cell proliferation, immune induction, wound healing, cytokine release, chemotaxis and protease-antiprotease balance. AMPs qualify as prototypes of innovative drugs that may be used as antimicrobials, anti-lipopolysaccharide drugs or modifiers of inflammation. Several strategies have been followed to identify lead candidates for drug development, to modify the peptides' structures, and to produce sufficient amounts for pre-clinical and clinical studies (Koczulla and Bals, 2003).

Antimicrobial peptides (AMPs) are small molecular weight proteins with broad spectrum antimicrobial activity against bacteria, viruses, and fungi. These evolutionarily conserved peptides are usually positively charged and have both a hydrophobic and hydrophilic side that enables the molecule to be soluble in aqueous environments y*et al*so enter lipid-rich membranes. Once in a target microbial membrane, the peptide kills target cells through diverse mechanisms. Cathelicidins and defensins are major groups of epidermal AMPs. Decreased levels of these peptides have been noted for patients with atopic dermatitis and Kostmann's syndrome, a congenital neutropenia. In addition to important antimicrobial properties, growing evidence indicates that AMPs alter the host immune response through receptor-dependent interactions. AMPs have been shown to be important in such diverse functions as angiogenesis, wound healing, and chemotaxis. As our knowledge of AMP biology expands, the precise role and relevance of these peptides will be better elucidated (Izadpanah and Gallo, 2005).

The rapid increase in drug-resistant infections has presented a serious challenge to antimicrobial therapies. The failure of the most potent antibiotics to kill "superbugs" emphasizes the urgent need to develop other control agents. Here we review the history

and new development of antimicrobial peptides (AMPs), a growing class of natural and synthetic peptides with a wide spectrum of targets including viruses, bacteria, fungi, and parasites. They summarize the major types of AMPs, their modes of action, and the common mechanisms of AMP resistance. In addition, they have discussed the principles for designing effective AMPs and the potential of using AMPs to control biofilms (multicellular structures of bacteria embedded in extracellular matrixes) and persister cells (Bahar and Ren, 2013).

Antimicrobial peptides (AMPs) are an important component of the natural defences of most living organisms against invading pathogens. These are relatively small (<10 kDa), cationic and amphipathic peptides of variable length, sequence and structure. During the past two decades several AMPs have been isolated from a wide variety of animals, both vertebrates and invertebrates, and plants as well as from bacteria and fungi. Most of these peptides are obtained from different sources like macrophages, neutrophils, epithelial cells, haemocytes, fat body, reproductive tract, etc. These peptides exhibit broad-spectrum activity against a wide range of microorganisms including Gram-positive and Gram-negative bacteria, protozoa, yeast, fungi and viruses. The peptides are broadly classified into five major groups namely (a) peptides that form α-helical structures, (b) peptides rich in cysteine residues, (c) peptides that form β-sheet, (d) peptides rich in regular amino acids namely histatin, arginine and proline and (e) peptides composed of rare and modified amino acids. Peptides, namely magainin and nisin have been shown to demonstrate contraceptive properties *in vitro* and *in vivo*. A few peptides have already entered clinical trials for the treatment of impetigo, diabetic foot ulcers and gastric helicobacter infections. In this review, they discussed the source, structures and mode of action with special reference to therapeutic considerations of various AMPs (Reddy *et al.,* 2004).

2.2.1 Importance of AMPs

Different classes of antimicrobial peptides (AMPs) found in the gastrointestinal (GI) tract of avian species, and their antimicrobial and immunomodulatory activities. The potential benefits of synthetic AMP in poultry production are examined, in the context of the use of AMP as alternatives to antimicrobial growth promoters (AGP). Since the mid-

1950s, antibiotic growth promoters (AGP) have been used in feed at low prophylactic doses to modulate the homoeostasis of intestinal microbiota, decreasing the risk of intestinal dysbacteriosis and the growth of pathogens within the avian gut. Over the last three decades, AGP have faced major regulatory restrictions due to concerns of generating antimicrobial resistance (AMR). It is now well documented that the rate of infectious disease outbreaks is higher in flocks that are not fed prophylactic antibiotics, resulting in a compensatory increase in antimicrobial use for therapeutic purposes. Endogenous natural AMP production is associated with the presence of microbiota and their interaction with the intestinal epithelial and lamina propria lymphoid cells. Their antimicrobial activity shapes the beneficial microbiota population and controls intestinal pathogens such *Clostridium* and *Salmonella* spp., and stimulates the development and maturation of the local immune system. Similar to AGP, AMP can establish a well-balanced gut beneficial microbiota for adequate immune competence, animal health and high growth performance parameters such as feed intake, daily weight, feed conversion and accumulated mortality. Antimicrobial proteins and peptides constitute an essential part of the innate immune system of all organisms and protect the host from invading pathogenic bacteria, viruses, fungi, and parasites by interacting with the negatively charged pathogen membranes (Nazeer *et al*., 2021).

Multiple challenges confront the increasing demand for wholesome poultry food products, including governmental restrictions on the use of antibiotic growth promoters (AGPs), nutritional requirements to obtain maximum growth potential, understanding cross talks among the immunity–microbiota–neuroendocrine system in the gut to maximize intestinal efficiency, high-density production conditions, waste management, and the emergence of infectious pathogens. Therefore, much interest has focused on the development of alternative, antibiotic-free methods of commercial poultry production. Initially, alternatives to antibiotics included any strategies that replace AGPs, but now include any feed additives or treatment that will allow antibiotic-free animal production to prevent and/or treat diseases. These newer disease control strategies can be classified broadly into those that are directly cytotoxic against infectious agents or remove pathogenic toxins, including vaccines, hyperimmune antibodies, antimicrobial peptides,

and bacteriophages, and those that augment non-specific host immunity and gut health, including phytochemicals, adjuvants, prebiotics, and probiotics. Furthermore, because the gut microbiota influences various physiological aspects of the immune response, brain function, and gut health, most antibiotic alternatives are expected to promote beneficial microbes that will benefit host physiological responses (Kim and Lillehoj, 2019).

Bacteriocins, antimicrobial peptides, and bacteriophage have attracted attention as potential substitutes for, or as additions to, currently used antimicrobial compounds. Bacteriocins are proteinaceous compounds of bacterial origin that are lethal to bacteria other than the producing strain. It is assumed that some of the bacteria in the intestinal tract produce bacteriocins as a means to achieve a competitive advantage, and bacteriocin-producing bacteria might be a desirable part of competitive exclusion preparations. Purified or partially purified bacteriocins could be used as preservatives or for the reduction or elimination of certain pathogens. Currently only nisin, produced by certain strains of *Lactococcus lactis* sub sp. lactis, has regulatory approval for use in certain foods, and its use for poultry products has been studied extensively. Exploration of the application of antimicrobial peptides from sources other than bacteria to poultry has not yet commenced to a significant extent. Evidence for the ability of chickens to produce such antimicrobial peptides has been provided, and it is likely that these peptides play an important role in the defense against various pathogens (Joerger, 2003).

The length of an AMP is important to its activity because at least 7–8 amino acids are needed to form amphipathic structures with hydrophobic and hydrophilic faces on opposite sides of a peptide molecule. Typically, cationic AMPs have more than two net charges ranging from +2 to +9 which makes it prone to bind to the negative membrane of bacteria and other microorganisms. And also, the cationic charge determines the selective binding of AMPs toward microbial membrane rather than host cell membrane, which is likely because the microbial membrane has higher overall negative charge compared to host cell membrane. Hydrophobicity has also been shown to influence the activity and selectivity of AMP molecules. Almost 50% of amino acids in the primary sequence of natural AMPs are hydrophobic residues (Shen *et al*., 2018).

2.2.2 Classification of AMPs

2.2.2.1 Classification of AMPs based on sources

2.2.2.1.1 Mammalian antimicrobial peptides

Mammalian antimicrobial peptides are found in human, sheep, cattle, and other vertebrates. Cathelicidins and defensins are the main families of AMPs. Defensins can be divided into α-, β-, and θ-defensins depending on the position of disulfide bonds. Human host defense peptides (HDPs) can protect human from microbial infections but show different expressions in every stage of human growth. For example, cathelicidin LL-37, a famous AMP derived from the human body, is usually detected in the skin of newborn infants, whereas human beta-defensin 2 (hBD-2) is often expressed in the elderly instead of the young (Gschwandtner *et al*., 2014).

2.2.2.1.2 Amphibian-derived antimicrobial peptides

Antimicrobial peptides from amphibians play an important role in the protection of amphibians from the pathogens that have induced the global amphibian population decline. Frogs are the main source of amphibian AMPs and the most famous AMP from frogs is magainin; the skin secretions of frogs from genera Xenopus, Silurana, Hymenochirus, and Pseudhymenochirus under the Pipidae family are rich in AMPs. Furthermore, cancrin, which has an amino acid sequence of GSAQPYKQLHKVVNWDPYG, has been reported as the first AMP from the sea amphibian Rana cancrivora (Lu *et al*., 2008).

2.2.2.1.3 Insect-derived antimicrobial peptides

Antimicrobial peptides are mainly synthesized in fat bodies and blood cells of insects, which is one of the main reasons for insects' strong adaptability to survival. Cecropin is the most famous family of AMPs from insects, and it can be found in guppy silkworm, bees, Drosophila. Cecropin A shows activity against different inflammatory diseases and cancers. What should be known is that the number of AMPs varies greatly between species, for example, invasive harlequin ladybird (*Harmonia axyridis*) and black soldier fly (*Hermetia illucens*) have up to 50 AMPs, while pea aphid (*Acyrthosiphon pisum*) lacks AMPs (Shelomi *et al*., 2020).

2.2.2.1.4 Microorganisms-derived antimicrobial peptides

Antimicrobial peptides can be obtained from microorganisms like bacteria and fungi, and some famous peptides are nisin, gramicidin from *Lactococcus lactis*, *Bacillus subtilis,* and *Bacillus brevis*. Due to the high price of chemical synthesis of AMPs, the biological expression has attracted the increase of attention. Specific yeast species like *Pichia pastoris*, *Saccharomyces cerevisiae*, and bacteria like *Escherichia coli*, *B. subtilis*, and plants have been used for expression systems, but it should be noticed that because of the toxicity, proteolytic degradation, and purification, AMPs are difficult to be produced in *E. coli*, which is necessary to take advantage of fusion tags (Yu *et al*., 2015). More marine-derived AMPs have been reported to have given the increasing value allotted by people to marine resources. Although most of the reported marine AMPs have been tested in vitro, several of these AMPs have shown promising results in vivo, for example, As-CATH4 shows an immunity-stimulating effect *in vivo* and can enhance the anti-infective capability of drugs used in combination with it (Semreen *et al*., 2018).

2.2.2.2 Classification based on activity

The activity of AMPs can be divided according to the statistics of the ADP3 database.

2.2.2.2.1 Antibacterial peptides

Antibacterial peptides account for a large part of AMPs and have a broad inhibitory effect on common pathogenic bacteria, such as VRE, *Acinetobacter baumannii*, and MRSA in clinical medicine and *S. aureus, Listeria monocytogenes*, *E. coli* in food and *Salmonella*, *Vibrio parahaemolyticus* in aquatic products. Many natural and synthetic AMPs like nisin, cecropins and defensins have shown good inhibition activity to Gram-positive bacteria and Gram-negative bacteria. In recent research, AMPs P5 (YIRKIRRFFKKLKKILKK-NH2) and P9 (SYERKINRHFKTLKKNLKKK-NH2), which are designed based on Aristicluthys nobilia interferon-I, inhibit MRSA and show a low cytotoxicity (Li *et al*., 2019).

2.2.2.2.2 Antifungal peptides (AFPs)

Antifungal peptides are a subclass of AMPs that address fungal infections with enhanced drug resistance. Many AFPs have shown excellent anti-fungal activities against common pathogenic fungi, such as *Aspergillus* and *Candida albicans* in clinical medicine, yeast, filamentous fungi (e.g., *Aspergillus flavus*), mold in food and agriculture. Except for brevinin, ranatuerin, cecropins, many synthetic peptides also show good antifungal activity. For example, AurH1, derived from aurein 1.2, can effectively treat *C. albicans* infection, which has a lethal rate up to 40% (Madanchi *et al*., 2020).

2.2.2.2.3 Antiviral peptides (AVPs)

Viruses cause serious harm to human life and huge economic losses to the animal husbandry. The COVID-19, which is the recent outbreak, has caused great loss of lives and properties. Furthermore, foot-and-mouth disease virus, avian influenza virus (AIV), and HIV are long-term threats to human life. So, it is extremely urgent to solve these problems, and antiviral peptides provide new ways. Antiviral peptides show a strong killing effect on viruses mainly by (1) inhibiting virus attachment and virus cell membrane fusion, (2) destroying the virus envelope, or (3) inhibiting virus replication (Jung *et al*., 2019)

2.2.2.2.4 Antiparasitic peptides

Parasitic protozoa can cause diseases in human and animals through a variety of routes, including animal-to-person or person-to-person contact, water, soil, and food. And with the increase in parasite drug resistance, the need for new treatments has increased. Antiparasitic peptides show their killing effect on parasites which cause diseases such as malaria and leishmaniasis and AMPs like cathelicidin, temporinsSHd show high inhibition activity against parasites (Abbassi *et al*., 2013).

2.2.2.2.5 Anticancer peptides (ACPs)

The ACPs show anticancer mechanisms by (1) recruiting immune cells (such as dendritic cells) to kill tumor cells, (2) inducing the necrosis or apoptosis of cancer cells, (3) inhibiting angiogenesis to eliminate tumor nutrition and prevent metastasis, and (4)

activating certain regulatory functional proteins to interfere with the gene transcription and translation of tumor cells (Wu *et al*., 2020; Ma *et al*., 2020).

2.2.2.3 Classification of AMPs based on amino acid-rich species

2.2.2.3.1 Proline-rich peptides (PrAMPs)

Proline is a typical non-polar amino acid. PrAMPs behave differently from other AMPs, that is, they enter bacterial cytoplasm by the inner membrane transporter SbmA instead of killing bacteria through membrane destruction. Once in the cytoplasm, PrAMPs target ribosomes and block the binding of aminoacyl-tRNA to peptidyltransferase center or trap decoding release factors on the ribosome during the termination of translation to interfere with protein synthesis (Seefeldt *et al*., 2015).

2.2.2.3.2 Tryptophan- and arginine-rich antimicrobial peptides

Tryptophan (Trp), as a non-polar amino acid, has a remarkable effect on the interface region of the lipid bilayer, whereas Arg, as a basic amino acid, confers peptide charge and hydrogen bond interactions, which are essential properties to combine with the bacterial membrane's abundant anionic component. And it seems that Trp residues play the role of natural aromatic activators of Arg-rich AMPs by ion-pair-π interactions, thereby promoting enhanced peptide-membrane interactions. In addition to indolicidin and Triptrpticin which both are famous AMPs that rich in Arg and Trp residues. Octa 2 (RRWWRWWR) is also a typical Trp- and Arg-rich AMP that inhibits Gram-negative *E. coli* and Pseudomonas aeruginosa and Gram-positive S. aureus. And short Trp- and Arg-rich AMPs designed based on bovine and murine lactoferricin have also shown strong inhibitory action against bacteria (Bacalum *et al*., 2017).

2.2.2.3.3 Histidine-rich peptides

Histidine is a common basic amino acid, and histidine-rich AMPs show good membrane permeation activity. HV2 is a histidinerich AMP designed based on RR(XH)2XDPGX(YH)2RR–NH2 (where X represents I, W, V, and F). This peptide increases the permeability of bacterial cell membranes to cause cell membrane rupture and death. In addition, HV2 inhibits bacterial movement in a concentration-dependent manner

and shows a strong anti-inflammatory effect by inhibiting the production of tumor necrosis factor α (TNF-α) (Dong *et al*., 2019).

2.2.2.3.4 Glycine-rich antimicrobial peptides

The R group of glycine is generally classified as a non-polar amino acid in biology. Glycine-rich AMPs, such as attacins and diptericins, widely exist in nature. These peptides contain 14% to 22% glycine residues, which have an important effect on the tertiary structure of the peptide chain. A glycine-rich AMP derived from salmonid cathelicidins activates phagocyte-mediated microbicidal mechanisms, which differ from the mechanism of conventional AMPs (Este *et al*., 2016).

2.2.3 Mechanisms of action

The recently developed peptide GL13K is used as an example to illustrate many of the discussed concepts. Cationic AMPs typically exhibit an amphipathic conformation, which allows increased interaction with negatively charged bacterial membranes. Peptides undergo changes in conformation and aggregation state in the presence of membranes; conversely, lipid conformation and packing can adapt to the presence of peptides. As a consequence, a single peptide can act through several mechanisms depending on the peptide's structure, the peptide:lipid ratio, and the properties of the lipid membrane. Therefore, once a peptide has reached the cell wall, cell membrane, or its internal target, the difference in mechanism of action on gram-negative and gram-positive bacteria may be less pronounced than formerly assumed (Bechinger, B., and Gorr, S. U. 2017).

Antimicrobial peptides encompass a wide variety of structural motifs. Many peptides have α-helical structures. The majority of these peptides are cationic and amphipathic but there are also hydrophobic α-helical peptides which possess antimicrobial activity. In addition, some β-sheet peptides have antimicrobial activity and even antimicrobial α-helical peptides which have been modified to possess a β-structure retain part of their antimicrobial activity. There are also antimicrobial peptides which are rich in a certain specific amino acid such as Trp or His. In addition, antimicrobial peptides exist with thio-ether rings, which are lipopeptides or which have macrocyclic Cys knots.

Alternatively, a necessary but not sufficient property of these peptides may be to be able to pass through the membrane to reach a target inside the cell. The interaction of these peptides with biological membranes is not just a function of the peptide but is also modulated by the lipid components of the membrane. It is not likely that this diverse group of peptides has a single mechanism of action, but interaction of the peptides with membranes is an important requirement for most, if not all, antimicrobial peptides (Epand and Vogel, 1999).

2.3 Molecular docking

Lead discovery through searching of ligand databases with molecular docking techniques represents an attractive alternative to high-throughput random screening. The size of commercial databases imposes severe computational constraints on molecular docking, compromising the level of calculational detail permitted for each putative ligand. They described an alternative philosophies for docking which effectively address this challenge. With respect to the dynamic aspects of molecular recognition, these strategies lie along a spectrum of models bounded by the Lock-and-Key and Induced-Fit theories for ligand binding. They explored the potential of a rigid model in exploiting species specificity and of a tolerant model in predicting absolute ligand binding affinity. Current molecular docking methods are limited primarily by their ability to rank docked complexes; we therefore place particular emphasis on this aspect of the problem throughout our validation of docking strategies (Gschwend *et al*., 1996).

As the structures of more and more proteins and nucleic acids become available, molecular docking is increasingly considered for lead discovery. Recent studies consider the hit-rate enhancement of docking screens and the accuracy of docking structure predictions. As more structures are determined experimentally, docking against homology-modelled targets also becomes possible for more proteins. With more docking studies being undertaken, the drug-likeness and specificity of docking hits is also being examined (Shoichet *et al*., 2002).

Docking methodology aims to predict the experimental binding modes and affinities of small molecules within the binding site of particular receptor targets and is

currently used as a standard computational tool in drug design for lead compound optimisation and in virtual screening studies to find novel biologically active molecules. The basic tools of a docking methodology include a search algorithm and an energy scoring function for generating and evaluating ligand poses. They present the search algorithms and scoring functions most commonly used in current molecular docking methods that focus on protein–ligand applications. Protein flexibility, multiple ligand binding modes and the free-energy landscape profile for binding affinity prediction are important and interconnected challenges to be overcome by further methodological developments in the docking field (Guedes *et al*., 2014).

Molecular docking methodology explores the behaviour of small molecules in the binding site of a target protein. As more protein structures are determined experimentally using X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, molecular docking is increasingly used as a tool in drug discovery. Docking against homology-modelled targets also becomes possible for proteins whose structures are not known. With the docking strategies, the druggability of the compounds and their specificity against a particular target can be calculated for further lead optimization processes. Molecular docking programs perform a search algorithm in which the conformation of the ligand is evaluated recursively until the convergence to the minimum energy is reached. Finally, an affinity scoring function, ΔG [U total in kcal/mol], is employed to rank the candidate poses as the sum of the electrostatic and van der Waals energies. The driving forces for these specific interactions in biological systems aim toward complementarities between the shape and electrostatics of the binding site surfaces and the ligand or substrate (Pagadala *et al*., 2017).

By means of virtual screening of small molecules databases it is possible to identify new potential inhibitors against a target of interest. Molecular docking is a computer simulation procedure to predict the conformation of a receptor-ligand complex. Each docking program makes use of one or more specific search algorithms, which are the methods used to predict the possible conformations of a binary complex. They described several molecular docking search algorithms, and the programs which apply such methodologies and also discuss how virtual screening can be optimized, describing

methods that may increase accuracy of the simulation process, with relatively fast docking algorithms (Dias *et al*., 2008)

Coinfection of *Mycoplasma gallisepticum* (MG) and *Escherichia coli* (*E. coli*) is frequently reported in poultry farms. Baicalin possess various pharmacological properties such as anti-inflammatory, anticancer, and antioxidant, etc. However, the protective effects of baicalin against coinfection of MG and *E. coli* are still elusive. Baicalin (450 mg/kg) treatment was started on day 13 after infection and continued for 5 d. Histopathological examination, qRT-PCR, ELISA, and molecular docking technique were used to evaluate the effects of baicalin on MG and *E. coli* coinfection in chicken lung and trachea. The results showed that coinfection caused severe lesions in the lung and tracheal tissues. However, baicalin treatment partially alleviated these lesions in coinfection group. Histopathological examination showed the alveolar spaces and mucosal layer thickening was restored and cilia gradually recovered with baicalin treatment compared in coinfection group and MG-infection group. Meanwhile, IL-17 singling pathway–related genes were significantly reduced ($P < 0.05$) in baicalin treatment group in lung, including IL-17C, TRAF6, NF-κB, CXCL1, CXCL2, MMP1, GM-CSF, and MUC5AC. The activities of cytokines and chemokines (CXCL1, CXCL2, MMP1, GMCSF, and MUC5AC) were decreased significantly ($P < 0.05$) in baicalin-treated group. The molecular docking of baicalin and NF-κB showed the highest fitness score and interaction (Wu *et al*., 2020).

Zoonotic and antimicrobial-resistant *Escherichia coli* (hereafter, *E. coli*) is a global public health threat which can lead to detrimental effects on human health. Ninety-four *E. coli* isolates were obtained from samples collected from different locations in Bangladesh, and the isolates were identified using conventional microbiological tests. Phenotypic disk diffusion tests using 20 antimicrobial agents were performed according to CLSI-EUCAST guidelines, and minimum inhibitory concentrations (MICs) were determined for a subset of samples. *E. coli* isolates showed high resistance to colistin (88.30%), ciprofloxacin (77.66%), trimethoprim/sulfamethoxazole (76.60%), tigecycline (75.53%), and enrofloxacin (71.28%). Additionally, the pathotype *eaeA* gene was confirmed in ten randomly selected *E. coli* isolates using primer-specific polymerase chain reaction (PCR).

The presence of *mcr-1* gene was confirmed using PCR and sequencing analysis in six out of ten *E. coli* isolates. Furthermore, sequencing and phylogenetic

analyses revealed a similarity between the catalytic domain of *Neisseria meningitidis* lipoolygosaccharide phosphoethanolamine transferase A (LptA) and MCR proteins, indicating that the six tested isolates were colistin resistant. Finally, the findings of the present study showed that *E. coli* isolated from chicken harbored *mcr-1* gene, and multidrug and colistin resistance. These findings accentuate the need to implement strict measures to limit the imprudent use of antibiotics, particularly colistin, in agriculture and poultry farms (Uddin *et al*., 2022).

MATERIAL AND METHODS

III MATERIAL AND METHODS

The study pertaining to the research entitled "Molecular Docking studies on interactions of Antimicrobial peptides with Bacterial targets" was carried out in the Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, CoA, GKVK - 560065. The materials and methods adopted for the entire study is listed in this section.

3.1 Screening of potential AMPs from the database

3.1.1 APD3 (Antimicrobial peptide database):

The antimicrobial peptide database is an original database initially online in 2003. The APD2 (2009 version) has been regularly updated and further expanded into the APD3. This database currently focuses on natural antimicrobial peptides (AMPs) with defined sequence and activity. It includes a total of 2619 AMPs with 261 bacteriocins from bacteria, 4 AMPs from archaea, 7 from protists, 13 from fungi, 321 from plants and 1972 animal host defense peptides. The APD3 contains 2169 antibacterial, 172 antiviral, 105 anti-HIV, 959 antifungal, 80 antiparasitic and 185 anticancer peptides. Newly annotated are AMPs with antibiofilm, antimalarial, anti-protist, insecticidal, spermicidal, chemotactic, wound healing, antioxidant and protease inhibiting properties. Identification of potential AMPs and screen of the AMPs based on the parameters which enhances the antimicrobial activity from the database (Wang *et al*., 2015).

3.2 Antibacterial activity prediction of AMPs

3.2.1 iAMPred:

Identification and designing of AMPs through wet lab experiments may be resource intensive. Thus, computational identification will supplement in identifying and designing new antimicrobial agents. By using the computational tool, the best candidate peptide can be identified prior to synthesis and testing against microbes. The user has to supply single or multiple peptide sequence to run the *i*AMPpred. The sequences must be supplied in FASTA format. Moreover, each sequence should contain only standard amino acid residues in a single letter code format. The output is provided in a tabular format as given

below. The table shows the probability with which the peptide sequences are predicted as antibacterial, antiviral and antifungal. If the probability is >0.5, then the test peptide sequence is said to be predicted in positive class and other wise negative class (Meher *et al*., 2017).

3.3 Generation of peptide structure from amino-acid sequence

A peptide specified by one-letter amino acid codes (capitalization does not matter). Clicking Apply brings up another dialog for specifying backbone φ (phi) and ψ (psi) angles and other parameters. One or more rows can be chosen with the mouse and set to values either entered manually or supplied for various types of secondary structure. Rows in the dialog can be chosen with the left mouse button. Ctrl-click toggles the state (chosen or not) of single line. A block can be chosen by dragging, or by clicking on the first (or last) line in the desired block and then Shift-clicking on its last (or first) line. Sidechain conformations will be taken from the specified Rotamer library. The rotamer at each position will be chosen as described for the command swapaa with the criteria cp: by fewest number of clashes, and if a tie, then the highest probability according to the rotamer library. The residues are added in N→C order, so only clashes with more N-terminal residues are evaluated. The peptide will be assigned the specified chain ID. Clicking Apply (or OK, which also dismisses the dialog) creates the peptide. Hydrogen atoms are not included. Backbone bond lengths and angles are taken from the Amber ff99 parameters. Sidechain bond lengths and angles are taken from the Amber parameter files all*94.lib (Pettersen *et al*., 2004).

3.4 Prediction of peptide toxicity

ToxinPred (http://crdd.osdd.net/ raghava/toxinpred/) is a unique *in silico* method of its kind, which will be useful in predicting toxicity of peptides/ proteins. In addition, it will be useful in designing least toxic peptides and discovering toxic regions in proteins. The development of ToxinPred will provide momentum to peptide/protein-based drug discovery (Gupta *et al*., 2013).

3.5 Retrieving the Poultry Bacterial targets from PDB.

PDB (https://www.rcsb.org/pdb) is the standard format for the structure files, can search for a protein molecule by typing its name or PDB ID in the box provided, for every structure in PDB there will be a unique ID. It will be a four-letter alphanumeric ID. The first letter will be a number between one to nine and rest indicates a protein name. When the protein name is provided in the text box, results are displayed showing the data such as Molecule Name, PDB text, Structural

domains, Ontology Terms etc. The particular information on a molecule can be obtained by clicking on the displayed results. There will be more than one structure for each protein such that based on the criteria given by (van *et al*., 1997) one structure was selected and downloaded by choosing' Download Files' option. The criteria follows:

1) Only structures of 2.0 A^0 resolution or better were included.
2) No mutation or single site mutation
3) X-ray crystallographic structure are preferred

Penicillin-Binding Protein 6 (PBP6) is considered as target for *E coli* which causes colibacillosis in poultry. The related protein data bank is taken from the RCSB protein data bank (PDB) entry was searched for PBP6. For molecular docking, the crystal structure of penicillin-binding protein 6 (PBP6) from E. coli (PDB ID: 3ITA) was chosen (Masood *et al*., 2021).

For *Salmonella* which causes salmonellosis in poultry, integral membrane protein lipooligosaccharide phosphoethanolamine transferase A (LptA) from *Neisseria meningitidis* is considered as target. while the 3D structure of LptA (PDB ID: 5FGN), the best template of MCR-1 of Salmonella was retrieved from the RCSB Protein Data Bank server (Uddin *et al*., 2022).

3.6 ADT Tool.

3.6.1 Preparation of protein structure

The crystal structure of two proteins of *E-coli* (3ITA) and *Solmonella* sp (5FGN) were retrieved from the Protein Data Bank (PDB). The PDB file contains protein, ligand and water oxygen atoms.

The PDBQT of the protein are prepared using the Autodock tools software downloaded from MGL tools. Select and delete all the heteroatoms present in the PDB file. The removal of water molecules, repairing missing atoms, adding polar hydrogen only, and subsequently adding the Kollman charges to the protein structure (Rauf *et al*., 2015).

3.6.2 Preparation of peptide structure

The 10 modelled 3D peptide structure need to be prepared. Load the peptide structure into ADT. Assign partial charges to peptide and then go to torsion tree to detect the root. Now select ligand (peptide) once more to detect torsion tree and to select number of torsions (that would be left to default) and save the output in PDBQT format (Rauf *et al*., 2015).

3.6.3 Preparation of grid box of a protein

We need to define the 3D search space in which peptide has to bind to the protein active site. Ideally, if we do not know the binding site, we will either define a box that encloses the whole protein or perhaps a specific region of the protein. In the research, to speed up the docking process, we defined a search space that encloses the known binding site. To define the Grid Box will draw a box with opposite faces coloured in red, green and blue. Fiddle with the dials and see how you can enclose regions of the protein. In this instance we will use a spacing (angstrom) of 1Å (this is essentially a scaling factor). So set this dial to 1.000. So that we all get consistent results, let us set the (x, y, z) center and the number of points in (x, y, z) dimension. Make a note of these values which is shown in 3.6.4 (Rauf *et al*., 2015).

3.6.4 Preparation of docking configuration file

Before we can perform the actual docking, prepare a text file providing all the details about the pdbqt files of protein and its ligand and grid box details (Rauf *et al*., 2015).

The input file should look like:

3ITA receptor

receptor = 3ita.pdbqt

ligand = ligand.pdbqt

out = results.pdbqt

center_x = 5.035

center_y = -12.594

center_z = 2.92

size_x = 46

size_y = 95

size_z = 98

exhaustiveness = 8

5FGN receptor

receptor = 5fgn.pdbqt

ligand = ligand.pdbqt

out = results.pdbqt

center_x = 38.749

center_y = -16.915

center_z = -19.345

size_x = 52

size_y = 45

size_z = 45

exhaustiveness = 8

This defines the protein (receptor), ligand, number of docking modes to generate. All the docked modes will be collated in a file defined by output.pdbqt.

3.6.5 Protein-peptide docking

Computational docking methods have proven to be useful in the discovery and design of small-molecule drugs. Similar efforts are being made in the field of peptide therapeutics. we use a program called Autodock Vina for docking. Autodock Vina is a fast docking algorithm that requires minimal user intervention. Run vina to perform the docking. We will keep a log of all the program output in a file log.txt. This will take a few minutes depending on how fast your computer is. Once the run is complete, it has two files all.pdbqt, which contains all the docked modes, and log.txt, which contains a table of calculated affinities based on AutoDock Vina's scoring function (Trott and Olson 2010). The best docked mode, according to AutoDock Vina, is the first entry in all.pdbqt. The applicability of this program is limited to short peptides (up to a few amino acids). They also require the user to manually place a peptide conformation within the binding site.

3.6.6 Docking results analysis

3.6.6.1 Receptor-ligand (peptide) interactions tools

Receptor-ligand (Peptide) interactions tools can be used to prepare and to analyze receptor binding sites, dock ligands and fragments, and analyze docking results that can be obtained from (file:///C:/Program%20Files/BIOVIA/Discovery%20Studio%202021/share/doc/DSV/DSV.htm#docds/tsrlinteractions/rcli_vit.htm).

3.6.6.1.1 BIOVIA® Discovery Studio

A common task in Discovery Studio is to view a binding site and ligand in the Graphics View to understand the interactions between them. The View Interactions tools allow you to characterize and visualize ligand binding sites within receptors and to analyze the interactions between receptor residues and bound ligands. It analyzes the interactions between a receptor and a ligand. It can be used to view the interactions in a receptor-ligand complex determined by experiment or obtained from a set of ligands docked with a receptor.

3.6.6.1.1.1 Display receptor-ligand interactions

Create a ligand monitor for analyzing interactions between the defined ligand and receptor. It shows the first interaction shell of the ligand with the receptor. The monitor is automatically updated based on changes to the chemistry. In addition, changes to the current defined ligand and receptor are also updated as you use the navigation buttons to browse the ligands or change the defined receptor. See non-bond interactions.

3.6.6.1.1.2 Show receptor-ligand interactions on a 2D diagram

Generate a 2D depiction of the defined ligand and the binding site residues of the defined receptor, including amino acid residues, waters, and metal atoms. Interactions, such as hydrogen bond, charge-charge, and Pi interactions between the surrounding residues and the ligand are also displayed. Solvent accessibility of the ligand atom and the amino acid residues are shown in light blue shading surrounding the atom or residue. Heavier shading indicates more exposure to solvent.

3.7 Modification of screened AMPs.

3.7.1 Modification of AMPs by increasing hydrophobicity.

Hydrophobicity, as another important parameter to determine the activity of AMPs, is usually defined as the percentage of hydrophobic residues in the peptide, which determines the distribution of AMPs in the hydrophobic core of the membrane. Through comparison and analysis of most AMPs, it is found that hydrophobic amino acid residues account for about 40–60% of the total number of amino acid residues. This indicates the hydrophobicity and antimicrobial activity of AMPs are closely related to a certain extent, changing hydrophobicity may be one of the effective ways to improve the antimicrobial activity. Through this example, demonstrates that the simplest and traditional approach to increasing the hydrophobicity of AMPs is to substitute hydrophobic amino acids with hydrophilic amino acids in the screened AMPS sequence, which (Black and Mould 1991). The modified AMPs details shown in Table 10. The evaluation of modified AMPs are done by performing molecular docking follows the same methodology as 3.6.5 with the retrieved bacterial targets and the results shown in the Table 11.

3.7.2 Modification of AMPs by end-tagging with hydrophobic moieties.

End-tagging of AMPs with hydrophobic amino acid stretches offers an interesting approach to achieve high, but selective, AMP activity. The terminal modifications with natural amino acids tagged with hydrophobic residues such as Tryptophan (W) has been proved to be a facile and effective strategy to improve the activity and stability of AMPs combined with limited toxicity because this approach can maximize the penetration of the hydrophobic core into the lipid bilayers, while preserving the primary sequence of AMPs without any additional post synthesis modifications. The biological relevance of these effects was demonstrated *ex vivo* and *in vivo* in porcine S. aureus skin infection models. In agreement with these findings, the binding of GKH17-WWW was much higher than that of GKH17 at bacteria-mimicking DOPE/DOPG membranes, as was peptide-induced liposome leakage (Pasupuleti *et al*., 2009). The modified AMPs list is shown in the Table 14. The evaluation of modified AMPs are done by performing molecular docking follows the same methodology as 3.6.5 with the retrieved bacterial targets and the results shown in the Table 15.

RESULTS AND DISCUSSION

IV RESULTS AND DISCUSSION

Colibacillosis and Salmonellosis are the most common bacterial diseases in poultry. The bacterial pathogens exhibiting antibiotic resistance. For this in recent days poultry industry is using AMPs as alternative of antibiotics to inhibit the pathogens. Computer-Aided Drug Design (CADD) is the most time, resource, and cost-effective method of producing a drug against the bacterial diseases. In this respect, the current work attempted to screen of AMPs from the database and use it against bacterial pathogens utilizing molecular docking technique which is performed in AutoDock Vina 1.1.2 version.

4.1 Screening of Database for AMPs.

Identification of 12 AMPs based on the physiochemical properties of antimicrobial peptides like length (≤10 in number), charge (+1 to +5) and hydrophobic residue (41% to 50%) from the Antimicrobial Peptide Database (APD3) is shown in the Table1.

Table 1. AMPs selected from APD3 Database.

Sl. No.	APD_ID	Sequence
1.	AP00141	RKKWFW
2.	AP00551	FRWWHR
3.	AP01210	PFKLSLHL
4.	AP01211	TPFKLSLHL
5.	AP01212	EPFKLSLHL
6.	AP01342	SVAGRAQGM
7.	AP01357	FFHLHFHY
8.	AP03168	WNWSKSF
9.	AP03260	VNCWGKH
10.	AP03296	YRLCCR
11.	AP03312	KPQAVFP
12.	AP03469	KFDLKVTIK

Similarly, Mustafa *et al*., 2019 reported that to identify the potential antimicrobial peptides (AMPs) that can target the spike protein of MERS-CoV, they suggest a two-stage computational methodology. Database screening of AMPs from the APD3 database based on physicochemical characteristics is the first stage. Since the virus membrane is negatively charged, they selected AMPs from APD3 according to the following criteria like length between 20 and 50 amino acids net charge of +1, and the peptides with unknown anti-MERS-CoV activity. They chose a few peptides from the literature that have been experimentally demonstrated to have anti-MERS-CoV effect in addition to the list of AMPs. These peptides serve as positive controls, and two of them, P9 and HR2P, will be assessed in contrast to their docking complexes with MERS-CoV in our expected complexes.

4.1.1 Prediction of antibacterial activity.

Prediction of antibacterial activity of the screened AMPs by using iAMPred tool is shown in Table 2.

Table 2. Antibacterial activity of screened AMPs.

Sl. No.	APD3_ID	Antibacterial (Probability value i.e.,1)
1.	AP00141	0.78
2.	AP00551	0.76
3.	AP01210	0.86
4.	AP01211	0.53
5.	AP01212	0.46
6.	AP01342	0.36
7.	AP01357	0.66
8.	AP03168	0.73
9.	AP03260	0.91
10.	AP03296	0.85
11.	AP03312	0.53
12.	AP03469	0.96

After prediction of antibacterial activity by subjecting AMP sequences in iAMPred tool, the AMPs of APD_ID (AP01212 and AP01342) have obtained the probability value of <0.5 are rejected. So out of 12 screened AMPs only 10 AMPs are potential to exhibit antibacterial property. All the ten AMPs sequences and its source (Table 3) from APD3 database and the AMPs are 3D structure modelled by using University of California, San Francisco - UCSF Chimera tool.

Table 3. Screened AMPs sequence and its Source

Sl. No.	APD_ID	Sequence	Name/Class	Source
1.	AP00141	RKKWFW	PAF26	Synthetic construct
2.	AP00551	FRWWHR	Combi-2	Synthetic construct
3.	AP01210	PFKLSLHL	Jelleine-I	*Apis mellifera*
4.	AP01211	TPFKLSLHL	Jelleine-II	*Apis mellifera*
5.	AP01357	FFHLHFHY	PL-101	*Styela plicata*
6.	AP03168	WNWSKSF	Darobactin A	Synthetic construct
7.	AP03260	VNCWGKH	Tryglysin B	*Streptococcus mutans* UA159
8.	AP03296	YRLCCR	HD5(26-32)	*Homo sapiens*
9.	AP03312	KPQAVFP	SyCPA 2	*Burkholderia gladioli* BSR3
10.	AP03469	KFDLKVTIK	Hs10	Human proteome, *Homo sapiens*

4.1.2 Prediction of toxicity for screened AMPs.

Toxicity of the antimicrobial peptides has been predicted by using the tool ToxinPred. The tool is a unique *in silico* method of its kind, which will be useful in predicting toxicity of ten screened peptides and all are resulted Non-Toxin with the negative score of Support Vector Machine (SVM) algorithm is shown in the Table 4.

4.1.3 Screened AMPs physiochemical properties and boman index.

For the screened AMPs, the physiochemical properties and the boman index are calculated by using the tool antimicrobial peptide calculator and predictor which is incorporated in the APD3 database is shown in the Table 5.

Table 4. Prediction of toxicity of screened AMPs

Sl. No.	APD_ID	Sequence	Mutation position	SVM score	Prediction	Molecular weight (Da)
1.	AP00141	RKKWFW	No Mutation	-0.77	Non-Toxin	950.23
2.	AP00551	FRWWHR	No Mutation	-1.11	Non-Toxin	987.21
3.	AP01210	PFKLSLHL	No Mutation	-0.98	Non-Toxin	954.30
4.	AP01211	TPFKLSLHL	No Mutation	-1.02	Non-Toxin	1055.42
5.	AP01357	FFHLHFHY	No Mutation	-0.54	Non-Toxin	1147.42
6.	AP03168	WNWSKSF	No Mutation	-1.15	Non-Toxin	954.54
7.	AP03260	VNCWGKH	No Mutation	-0.46	Non-Toxin	843.03
8.	AP03296	YRLCCR	No Mutation	-0.37	Non-Toxin	813.07
9.	AP03312	KPQAVFP	No Mutation	-1.08	Non-Toxin	786.03
10.	AP03469	KFDLKVTIK	No Mutation	-1.02	Non-Toxin	1091.49

Table 5. Features of screened AMPs

Physiochemical properties of AMPs					Boman Index (Kcal/mol)
Sl. No.	APD_ID	Length (≤10)	Net charge (+1 to +5)	Hydrophobic residue%	
1.	AP00141	6	3	50	3.06
2.	AP00551	6	3	50	4.47
3.	AP01210	8	3	50	-0.51
4.	AP01211	9	3	44	-0.17
5.	AP01357	8	1	50	0.03
6.	AP03168	7	1	42	1.62
7.	AP03260	7	2	43	1.18
8.	AP03296	6	2	50	3.75
9.	AP03312	7	1	43	0.32
10.	AP03469	9	3	44	1.23

4.2 Retrieval of target structure

Protein 3D structure of the bacterial diseases like colibacillosis and Salmonellosis are downloaded from RCSB PDB database (http://www.rcsb.org/), in PDB format are given in the Table 6.

Table 6. Targets of poultry bacterial pathogens

Sl. No.	Pathogen	PDB_ID	Resolution (A^0)	No. of Chains	Sequence length	Mutation
1.	*E.coli*	3ITA receptor	1.8	A, B, C, D	352	0
2.	*Solmonella*	5FGN receptor	2.75	A	550	0

After retrieval of X-ray structure of PBP 6 protein and LptA protein from PDB database with the PDB ID: 3ITA and 5FGN which is prefusion structure of PBP 6 protein and LptA protein respectively. In the 3ITA receptor structure four chains are present, in this chain A is considered as receptor. Preparing the grid box for both 3ITA receptor and 5FGN receptor is follows the same methodology as given in the 3.6.3 and 3.6.4 which is shown in the Fig. 1 and Fig. 2.

Similarly, Mustafa *et al*., 2019 reported that they retrieved the prefusion structure of the MERSCoV spike glycoprotein's cryo-EM structure from Protein Data Bank (PDB), which has the PDB ID: 5X59. The structure of the MERS-CoV spike protein postfusion was determined by electron microscopy at a resolution of $3.7A^0$. This structure has 3969 amino acid residues and a total mass of 444204.84 KDa.

4.2.1 Molecular docking of AMPs against 3ITA receptor and 5FGN receptor.

From grid output file the configuration file “conf.txt” was prepared and command prompt was used for ADT molecular docking by giving command “program files\the scripps research institute\vina\vina. exe - config conf.txt - log log.txt” It generated the output file with the docking score or binding affinity (Kcal/mol), similarly were studied with the help of AutoDock Vina 1.1.2 version, and their binding affinities are showed in Table 7.

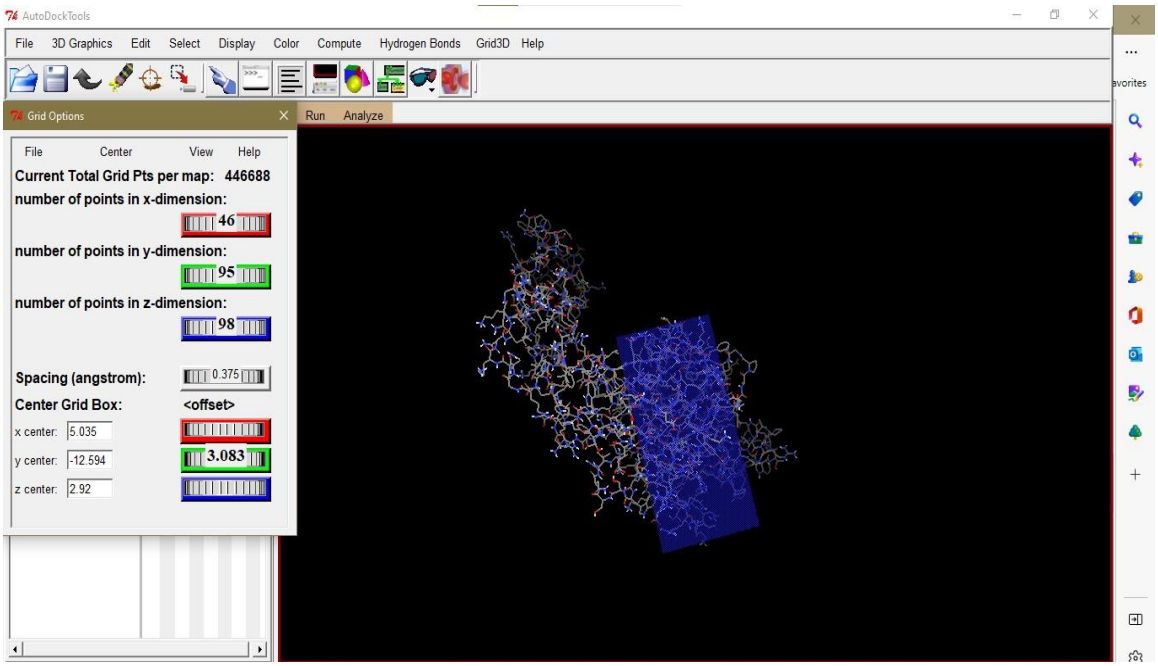

Figure 1: Shows the Grid Box Over the 3ITA receptor for docking in the ADT software

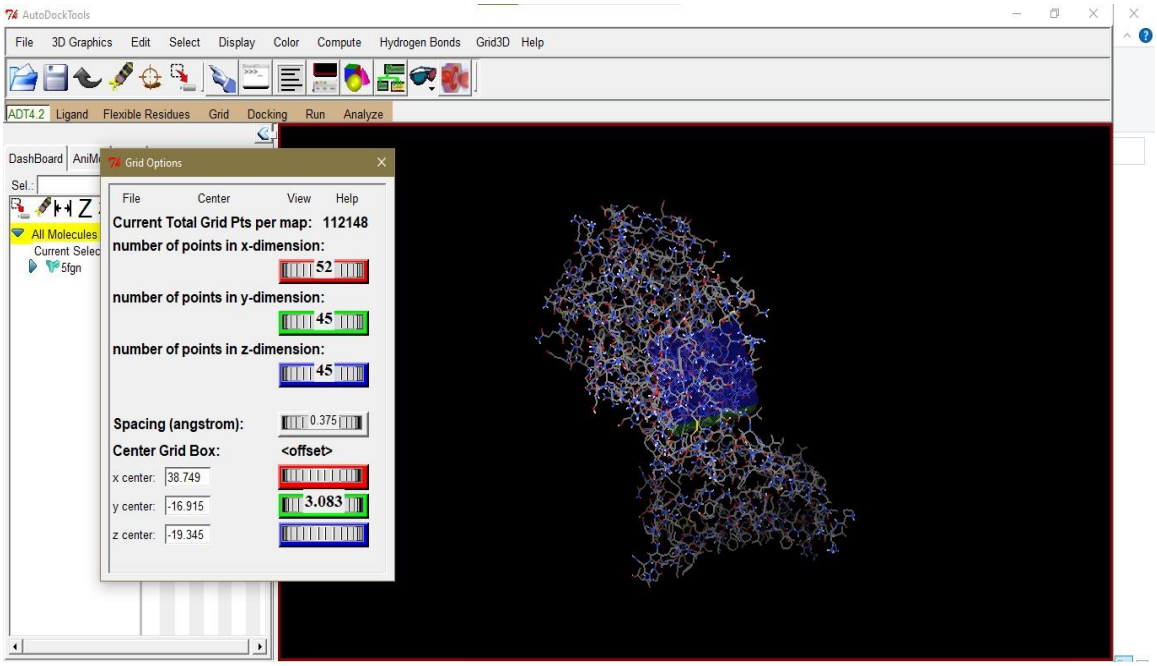

Figure 2: Shows the Grid Box Over the 5FGN receptor for docking in the ADT software

The results revealed that all the AMPs showed better binding affinity ranging from −6.0 to −8.7 Kcal/mol for *E coli* target 3ITA receptor and also for *Salmonella* target 5FGN receptor of better binding affinity ranging from −6.8 to −9.4 Kcal/mol. Among them, APD ID (AP00551) found to be best with −8.7 Kcal/mol at 3ITA receptor (Fig. 3), penicillin-binding protein of *E. coli* and APD ID (AP01357) found to be best with −9.4 Kcal/mol at lipid A phosphoethanolamine transferase for Salmonellosis (Fig. 6). Residues involved in interaction between 3ITA receptor and AP00551 (shown in the Table 7) are obtained by using BIOVIA® Discovery Studio and also generates 3D (Fig. 4), 2D images (Fig. 5). Residues involved in interaction between 5FGN receptor and AP01357 (shown in the Table 8) are obtained by using BIOVIA® Discovery Studio and also generates 3D (Fig. 7), 2D images (Fig. 8).

According to the BIOVIA® Discovery Studio which generates the 2D image (Fig. 5) which describes AMP of AP00551 forms hydrogen bond interactions with SER A:40 and THR A:212 of 3ITA receptor and also forms covalent bond interactions with SER A:40 of 3ITA receptor. These residues may be considered as critical residues. AMP of AP01357 forms carbon hydrogen bond interactions with HIS A:189 and GLY A:198 of 5FGN receptor and also forms alkyl ion interaction with VAL A;64 of 5FGN receptor (Fig. 8). These residues may be considered as critical residues.

Similarly, Mustafa *et al*., 2019 reported that In order to do AMPs-Receptor Docking, which determines the binding mechanism of the shortlisted AMPs with the spike protein receptor 5X59 and the model AMPs, they used the piper module of the Schrodinger software. The AMPs are recognized as ligands and bound with receptor 5X59 for peptide-protein docking. Putative anti-MERS-CoV AMPs are chosen AMPs that outperform experimentally proven peptides against MERS-CoV in terms of Piper cluster size. Additionally, they used ClusPro 2.0 to look for protein-protein interactions. In order to map the interaction of the resulting docked complex, the docked complex structure output format was submitted to the Protein Interactions Calculator webpage. When determining the strength of the contact, various elements are taken into account, including the number of hydrogen bonds, hydrophobic residues, aromatic, and ionic contacts.

Table 7. Binding affinities of docked screened AMPs with the target receptors.

Sl. No.	AMPs	Binding Energies (Kcal/mol)	
		3ITA receptor	5FGN receptor
1.	AP00141	-7.9	-7,9
2.	AP00551	-8.7	-8.6
3.	AP01210	-7.4	-7.2
4.	AP01211	-6.3	-7.1
5.	AP01357	-7.0	-9.4
6.	AP03168	-6.0	-8.4
7.	AP03260	-6.6	-7.1
8.	AP03296	-7.2	-7.1
9.	AP03312	-6.0	-7.3
10.	AP03469	-6.2	-6.8

Table 8. Residue interaction between receptor and AMP complex

Sl. No.	Receptor	AMP ID of APD 3	Amino acid residues of receptor protein
1.	3ITA receptor	AP00551	TRP 4, HIS 5, ARG 6, SER 40, ARG 194, THR 212, ARG 244
2.	5FGN receptor	AP01357	VAL 64, HIS 189, GLY 198

From the PIC server AP00225 forms hydrophobic interactions with the amino acid residues like Val790, Tyr1142, Phe764, Leu731, Ile768, Pro1143, Pro767, and Val770; hydrogen bond interactions with Pro730; and ionic interactions with Gln792 and Ser734. AP00180 forms hydrophobic interactions with the amino acid residues like Ala1007, Val790, Leu731, Pro767, Ile768, and Tyr1142; hydrogen bond interactions with Gly789 and Pro730; and ionic interactions with Glu1017 and Asp740. AP00549 forms hydrophobic interactions with the amino acid residues like Ala1049, Pro59, Tyr64, Tyr928, Val929, Ala930, Ala920, Ile69, and Tyr71; hydrogen bond interactions with Ala1049 and Gly61; and ionic interactions with Arg1057, Arg62, and Asp922. AP00744 forms hydrophobic interactions with the amino acid residues like Leu1200, Pro767, Val1168, Ile1180, Leu780, Phe778, Pro1143, Val983, and Ile985; hydrogen bond interactions with Ala1206; and ionic interactions with Asp771. These residues may be considered as critical residues. Anti-MERS-CoV peptide P9, which has been experimentally confirmed, overlaps with residues in AP00549, while HR2P shares residues with AP00225, AP00180, and AP00744. The binding of the peptide AP00549 shares the same binding area as P9, as do the binding of AP00225, AP00180, and AP00744. The following peptides and their ligands bind to the receptor spike: AP00179, AP00260, AP00340, AP02733, P9, and HR2P. They get to the conclusion that by measuring the binding energy, molecular docking studies help to understand the antiviral activity of medications. Similarly in this research we screened ten AMPs with appropriate antibacterial activity based on the physiochemical properties of antimicrobial peptides like length (≤10 in number), charge (+1 to +5) and hydrophobic residue (41% to 50%) from the Antimicrobial Peptide Database (APD3) (Wang *et al*., 2015). The 10 AMPs docked with the poultry bacterial targets of 3ITA receptor (*E coli*) and 5FGN receptor (*Salmonella*). Among them, AP00551 found to be best with −8.7 Kcal/mol at 3ITA receptor, penicillin-binding protein of *E. coli* AND AP01357 found to be best with −9.4 Kcal/mol at lipid A phosphoethanolamine transferase for Salmonellosis. Residues involved in interaction between 3ITA receptor and AP00551 are TRP 4, HIS 5, ARG 6, SER 40, ARG 194, THR 212, ARG 244and Residues involved in interaction between 5FGN receptor and AP01357 are VAL 64, HIS 189, GLY 198.

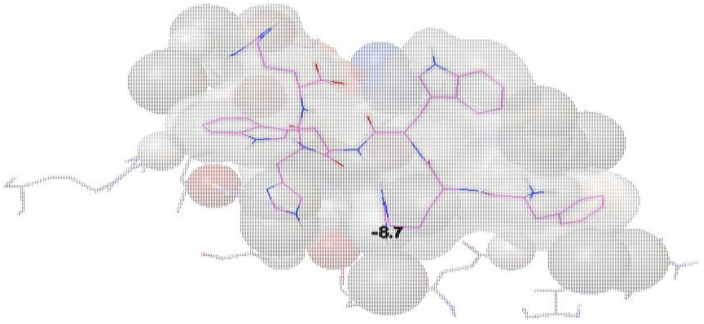

Figure 3: Docking result of 3ITA receptor and AMP(AP00551)

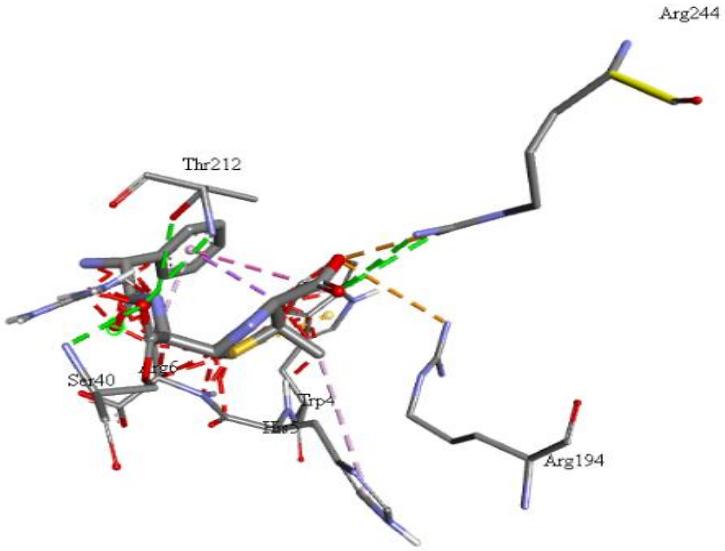

Figure 4: 3D image of interacting residues of 3ITA receptor and AMP (AP00551)

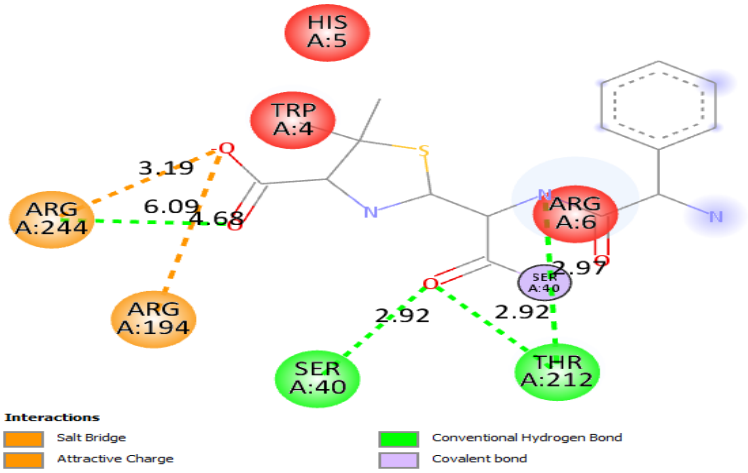

Figure 5: 2D image of interacting residues of 3ITA receptor and AMP (AP00551)

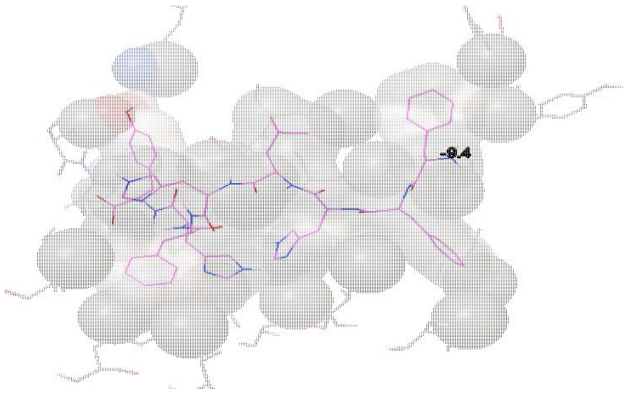

Figure 6: Docking result of 5FGN receptor and AMP (AP01357)

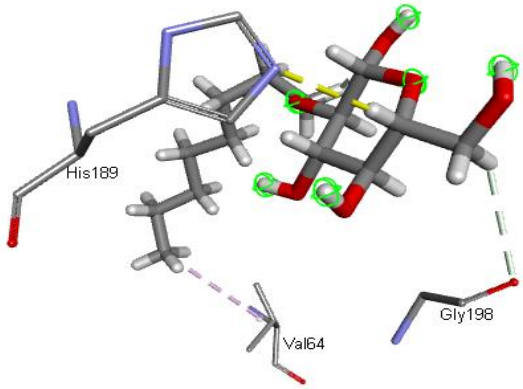

Figure 7: 3D image of interacting residues of 5FGN receptor and AMP (AP01357)

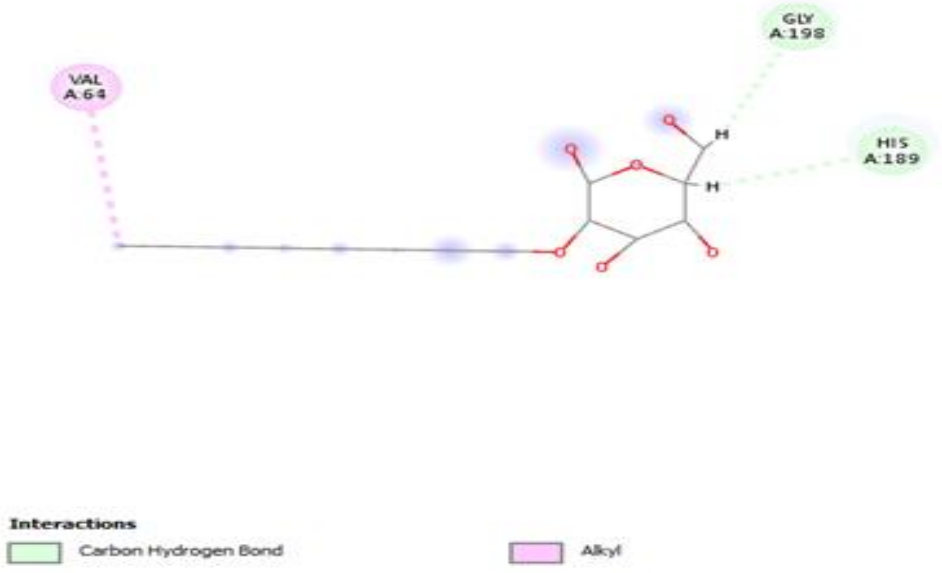

Figure 8: 2D image of interacting residues of 5FGN receptor and AMP (AP01357)

4.3 Modification of screened AMPs.

The antimicrobial activity of AMPs is affected by a variety of structural parameters, such as net charge, hydrophobicity, amphipathicity, and structural propensity. All parameters are interrelated, and antimicrobial activity and selectivity are the results of a delicate balance between them. Therefore, in recent decades, to optimize the overall properties of AMPs, a single factor modification to adjust the main parameters has been used. Great efforts have been made to explore the structure-function relationships of AMPs, researchers modified AMPs isolated from natural sources or synthesized to achieve the purpose of improving antimicrobial activity and reducing toxicity.

4.3.1 Modification of screened AMPs by increasing hydrophobicity.

High AMP adsorption, membrane rupture, and antibacterial effect can sometimes be facilitated by increasing the AMP charge density. However, due to electrostatic screening, highly charged and hydrophilic peptides lose much of their effect at high ionic strength. By increasing AMP hydrophobicity, sensitivity to these effects can be reduced (Ringstad *et al*., 2007). Phenylalanine is shown as the most hydrophobic amino acid (Black and Mould, 1991). Replacing Hydrophilic amino acids with Hydrophobic amino acid i.e., Phenylalanine (I) in the screened AMPs.

4.3.1.1 Toxicity prediction of modified AMPs.

Toxicity of the antimicrobial peptides has been predicted by using the tool ToxinPred. The tool is a unique *in silico* method of its kind, which will be useful in predicting toxicity of 10 modified peptides and all are resulted Non-Toxin except the sequence (IILCCI) obtained positive SVM score of 0.32. Then the tool modified toxin peptide into non-toxin by substituting alanine (A) in the 4^{th} position of the sequence of -0.67 SVM score which is shown in Table 9

4.3.1.2 AMPs physiochemical properties and Boman index.

For the screened modified AMPs, the physiochemical properties and the boman index are calculated by using the tool antimicrobial peptide calculator and predictor which is incorporated in the APD3 database which is shown in the Table 10.

Table 9. Prediction of toxicity of Modified AMPs

Sl. No.	Sequence	Mutation position	SVM score	Prediction	Molecular weight (Da)
1.	IIIWFW	No Mutation	-0.71	Non-Toxin	877.19
2.	FIWWII	No Mutation	-0.32	Non-Toxin	877.19
3.	PFILILIL	No Mutation	-0.87	Non-Toxin	941.40
4.	IPFILILIL	No Mutation	-0.82	Non-Toxin	1054.58
5.	FFILIFII	No Mutation	-0.88	Non-Toxin	1025.27
6.	WIWIIIF	No Mutation	-0.45	Non-Toxin	990.37
7.	VICWGII	No Mutation	-0.65	Non-Toxin	803.14
8.	IILCCI / IILACI	No Mutation / Mutation	0.32/-0.67	Toxin / Non-Toxin	644.96
9.	IPIAVFP	No Mutation	-1.01	Non-Toxin	756.05
10.	IFILIVIII	No Mutation	-0.94	Non-Toxin	1056.60

Table 10. Modified AMPs features

Sl. No.	APD3 Sequences	Modified Sequence	Hydrophobic Residue (%)	Net charge	Boman Index (Kcal/mol)
1.	RKKWFW	IIIWFW	100	0	-3.73
2.	FRWWHR	FIWWII	100	0	-3.73
3.	PFKLSLHL	PFILILIL	88	0	-4.06
4.	TPFKLSLHL	IPFILILIL	89	0	-4.15
5.	FFHLHFHY	FFILIFII	100	0	-4.19
6.	WNWSKSF	WIWIIIF	100	0	-3.9
7.	VNCWGKH	VICWGII	86	0	-3.33
8.	YRLCCR	IILACI	100	0	-3.79
9.	KPQAVFP	IPIAVFP	71	0	-2.66
10.	KFDLKVTIK	IFILIVIII	100	0	-4.6

4.3.1.3 Molecular Docking of modified AMPs against 3ITA receptor and 5FGN receptor

All the ten Modified AMPs are builded 3D form by using UCSF Chimera tool are subjected to docking with the help of AutoDock Vina 1.1.2 version, and their binding affinities are showed in Table 11. The results revealed that all the AMPs showed better binding affinity ranging from −5.9 to −7.0 Kcal/mol for *E coli* target 3ITA receptor and also for *Salmonella* target 5FGN receptor of better binding affinity ranging from −7.4 to −8.7 Kcal/mol. Among them, modified AMP sequence IPIAVFP (Fig. 9) found to be best with −7.0 Kcal/mol for 3ITA receptor of *E. coli* and the modified AMP sequence FIWWII (Fig. 12) found to be best with −8.7 Kcal/mol for 5FGN receptor of Salmonellosis.

Residues involved in interaction (shown in Table 12) between 3ITA receptor and modified AMP sequence IPIAVFP are obtained by using BIOVIA® Discovery Studio and also obtain 3D (Fig. 10), 2D images (Fig. 11). Residues involved in interaction (shown in Table 12) between 5FGN receptor and FIWWII are obtained by using BIOVIA® Discovery Studio and obtain 3D (Fig. 13), 2D images (Fig. 14).

Table 11. Binding affinities of docked modified AMPs with the target receptors.

Sl. No.	Modified Sequence	Binding Energies (Kcal/mol)	
		3ITA receptor	5FGN receptor
1.	IIIWFW	-6.9	-8.6
2.	FIWWII	-5.9	-8.7
3.	PFILILIL	-6.4	-7.5
4.	IPFILILIL	-6.5	-7.5
5.	FFILIFII	-6.7	-7.8
6.	WIWIIIF	-6.1	-8.0
7.	VICWGII	-6.3	-8.3
8.	IILACI	-6.6	-7.4
9.	IPIAVFP	-7.0	-8.1
10.	IFILIVIII	-6.4	-7.5

Table 12. Residue interaction between Receptor and modified AMP complex

Sl. No	Receptor	AMP sequence	Amino acid residues of receptor protein
1.	3ITA receptor	IPIAVFP	ALA 4, VAL 5, PHE 6, PRO 7, SER 40, ARG 194, THR 212, ARG 244
2.	5FGN receptor	FIWWII	VAL 64, HIS 189, GLY 198

According to the BIOVIA® Discovery Studio which generates the 2D image (Fig. 11) which describes modified AMP sequence FIWWII forms hydrogen bond interactions with SER A:40, PRO A:7 and THR A:212 and also forms covalent bond interactions with SER A:40. These residues may be considered as critical residues. Modified AMP sequence IPIAVFP forms carbon hydrogen bond interactions with HIS A:189 and GLY A:198 and also forms alkyl ion interaction with VAL A;64 is shown in the Fig. 12. These residues may be considered as critical residues.

4.3.2 Modification of screened AMPs by end-tagging with hydrophobic moieties.

End-tagging of AMPs with hydrophobic amino acid stretches offers an interesting approach to achieve high, but selective, AMP activity. The terminal modifications with natural amino acids tagged with hydrophobic residues such as Trp (W) has been proved to be a facile and effective strategy to improve the activity and stability of AMPs combined with limited toxicity because this approach can maximize the penetration of the hydrophobic core into the lipid bilayers, while preserving the primary sequence of AMPs without any additional post synthesis modifications.

4.3.2.1 Toxicity prediction of modified AMPs with W end-tagging.

Toxicity of the antimicrobial peptides has been predicted by using the tool ToxinPred. The tool is a unique *in silico* method of its kind, which will be useful in predicting toxicity of 10 modified peptides and all are resulted Non-Toxin by obtaining negative SVM score which is shown in Table 13.

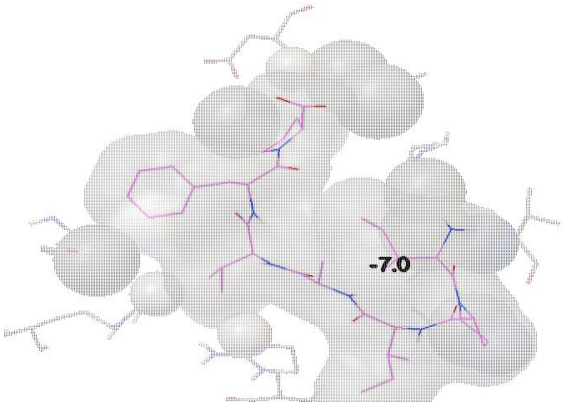

Figure 9: Docking result of 3ITA receptor and AMP (IPIAVFP)

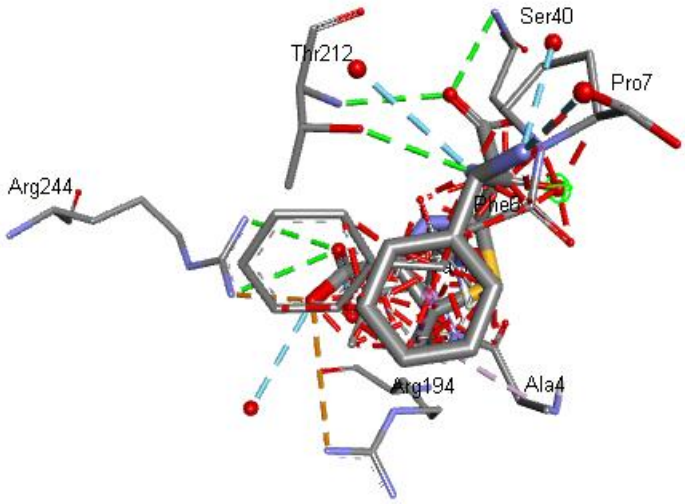

Figure 10: 3D image of interacting residues of 3ITA receptor and AMP (IPIAVFP)

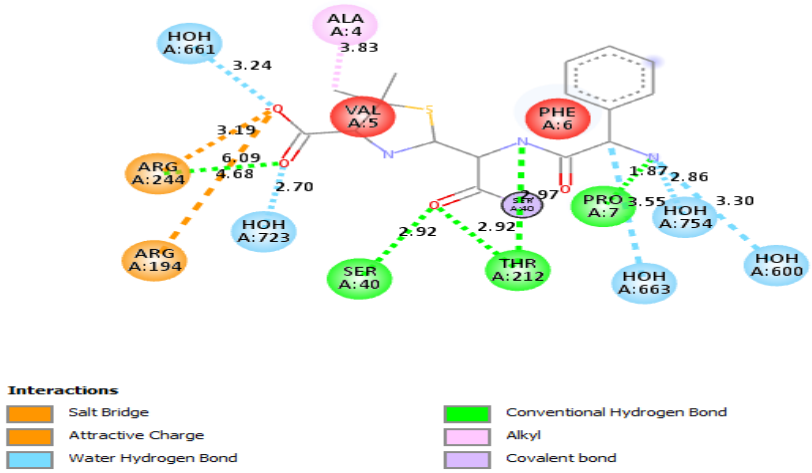

Figure 11: 2D image of interacting residues of 3ITA receptor and AMP (IPIAVFP)

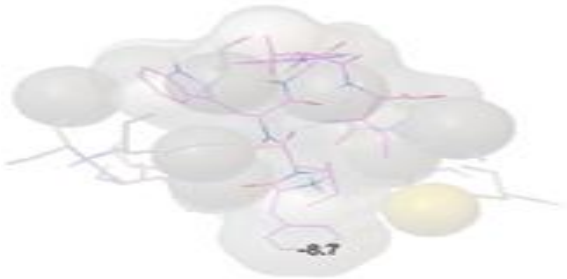

Figure 12: Docking result of 5FGN receptor and AMP (FIWWII)

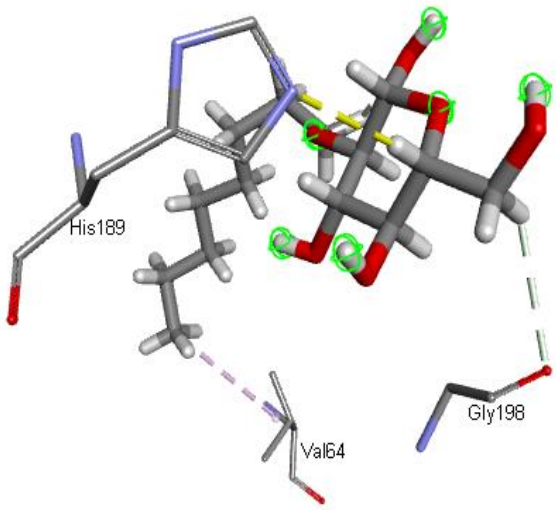

Figure 13: 3D image of interacting residues of 5FGN receptor and AMP (FIWWII)

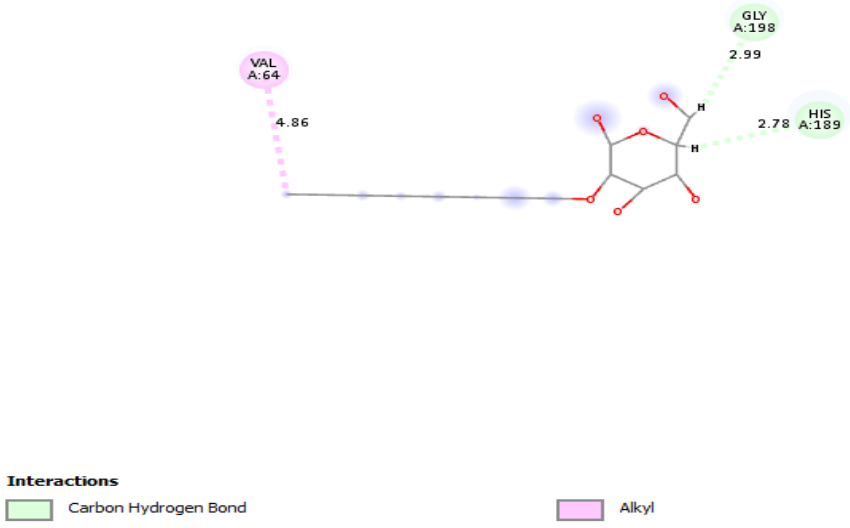

Figure 14: 2D image of interacting residues of 5FGN receptor and AMP (FIWWII)

Table 13. Prediction of toxicity of modified AMPs with W end-tagging

Sl. No.	Sequence	Mutation position	SVM score	Prediction	Molecular weight (Da)
1.	RKKWFWWWW	No Mutation	-0.89	Non-Toxin	1508.92
2.	FRWWHRWWW	No Mutation	-0.99	Non-Toxin	1545.90
3.	PFKLSLHLWWW	No Mutation	-0.96	Non-Toxin	1512.99
4.	TPFKLSLHLWWW	No Mutation	-0.99	Non-Toxin	1614.11
5.	FFHLHFHYWWW	No Mutation	-0.64	Non-Toxin	1706.11
6.	WNWSKSFWWW	No Mutation	-1.21	Non-Toxin	1512.83
7.	VNCWGKHWWW	No Mutation	-0.52	Non-Toxin	1401.76
8.	YRLCCRWWW	No Mutation	-0.25	Non-Toxin	1371.76
9.	KPQAVFPWWW	No Mutation	-0.99	Non-Toxin	1344.72
10.	KFDLKVTIKWWW	No Mutation	-0.92	Non-Toxin	1650.18

4.3.2.2 Physiochemical properties and boman index of Modified AMPs with W end-tagging.

For the screened modified AMPs with W end-tagging, the physiochemical properties and the boman index are calculated by using the tool antimicrobial peptide calculator and predictor which is incorporated in the APD3 database which is shown in the Table 14.

Table 14. Modification with W end-tagged AMPs features

Sl. No.	Modified Sequence	Length	Hydrophobic residue%	Net charge	Boman Index (Kcal/mol)
1.	RKKWFWWWW	9	67	3	1.26
2.	FRWWHRWWW	9	67	2.25	2.2
3.	PFKLSLHLWWW	11	64	1.25	-1.01
4.	TPFKLSLHLWWW	12	58	1.25	-0.71
5.	FFHLHFHYWWW	11	64	0.75	-0.61
6.	WNWSKSFWWW	10	60	1	0.43
7.	VNCWGKHWWW	10	60	1.25	0.12
8.	YRLCCRWWW	9	67	2	1.72
9.	KPQAVFPWWW	10	60	1	-0.47
10.	KFDLKVTIKWWW	12	58	2	0.34

4.3.3 Molecular docking of end-tagging AMPs against 3ITA receptor and 5FGN receptor.

All the 10 Modified AMPs are builded 3D form by using UCSF Chimera tool are subjected to docking with the help of AutoDock Vina 1.1.2 version, and their binding affinities are showed in Table 15. The results revealed that all the AMPs showed better binding affinity ranging from −5.9 to −8.6 Kcal/mol for *E coli* target 3ITA receptor and also for *Salmonella* target 5FGN receptor of better binding affinity ranging from −7.0 to −9.0 Kcal/mol. Among them, AMP sequence FFHLHFHYWWW (Fig. 15) found to be best with −8.6 Kcal/mol at 3ITA receptor, penicillin-binding protein of *E. coli* and the AMP sequence KPQAVFPWWW (Fig. 18) found to be best with −9.0 Kcal/mol at lipid A phosphoethanolamine transferase for Salmonellosis shown in Table 15. Residues involved in interaction between 3ITA receptor and AMP sequence FFHLHFHYW are obtained by using BIOVIA® Discovery Studio (shown in Table 16) and also generates 3D (Fig. 16), 2D images (Fig. 17). Residues involved in interaction between 5FGN receptor and AMP sequence KPQAVFPW are obtained by using BIOVIA® Discovery Studio (shown in Table 16) and also generates 3D (Fig. 19), 2D images (Fig. 20).

Table 15. Binding affinities of docked W-end tagged AMPs with target receptors

Sl. No.	Modified Sequence (end)	Binding Energies (Kcal/mol)	
		3ITA receptor	5FGN receptor
1.	RKKWFWWWW	-7.0	-8.0
2.	FRWWHRWWW	-6.3	-7.0
3.	PFKLSLHLWWW	-7.5	-8.2
4.	TPFKLSLHLWWW	-7.3	-7.4
5.	FFHLHFHYWWW	-8.6	-8.2
6.	WNWSKSFWWW	-7.4	-8.6
7.	VNCWGKHWWW	-7.1	-8.9
8.	YRLCCRWWW	-8.1	-7.7
9.	KPQAVFPWWW	-7.7	-9.0
10.	KFDLKVTIKWWW	-5.9	-7.8

Table 16. Residue interaction between Receptor and W-end tagged AMP complex.

Sl. No.	Receptor	AMP sequence	Amino acid residues of receptor protein
1.	3ITA receptor	FFHLHFHYWWW	THY 8, TRP 9, TRP 10, SER 40, ARG 194, THR 212, ARG 244
2.	5FGN receptor	KPQAVFPWWW	VAL 64, HIS 189, GLY 198

According to the BIOVIA® Discovery Studio which generates the 2D image (Fig. 17) which describes modified AMP sequence (FFHLHFHYWWW) forms hydrogen bond interactions with SER A:40, THY A:8 and THR A:212 and also forms covalent bond interactions with SER A:40. These residues may be considered as critical residues. Modified AMP sequence KPQAVFPWWW forms carbon hydrogen bond interactions with HIS A:189 and GLY A:198 and also forms alkyl ion interaction with VAL A;64 shown in Fig. 20. These residues may be considered as critical residues.

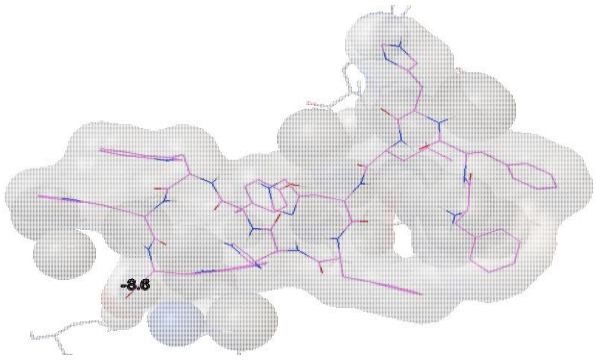

Figure 15: Docking result of 3ITA receptor and AMP (FFHLHFHYWWW)

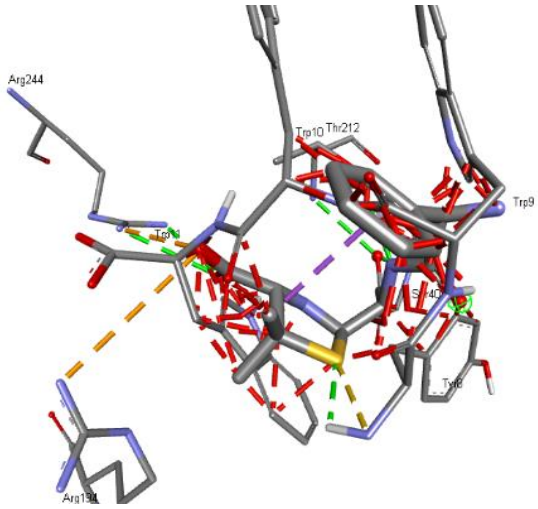

Figure 16: 3D image of interacting residues of 3ITA receptor and AMP (FFHLHFHYWWW)

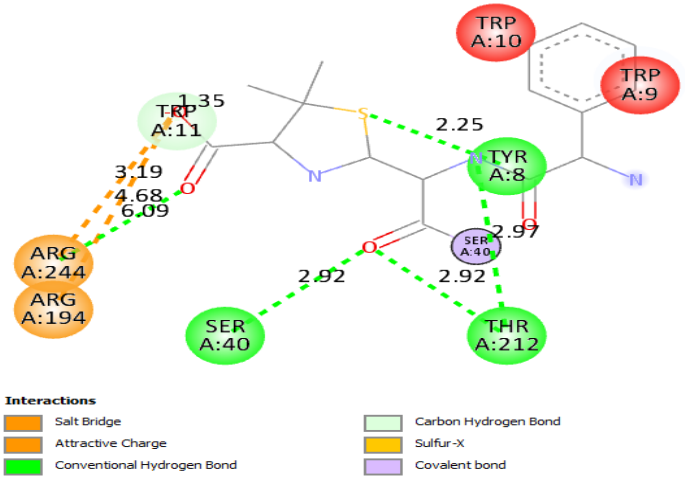

Figure 17: 2D image of interacting residues of 3ITA receptor and AMP (FFHLHFHYWWW)

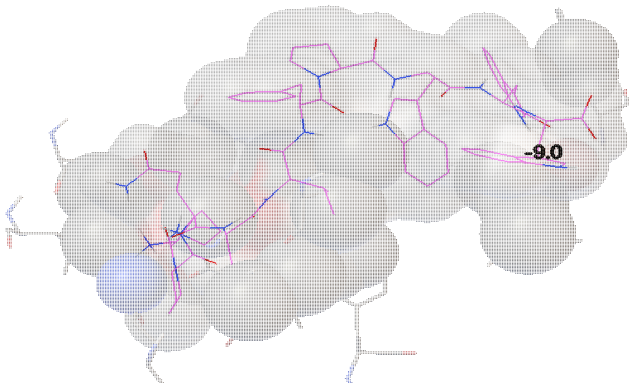

Figure 18: Docking result of 5FGN receptor and AMP (KPQAVFPWWW)

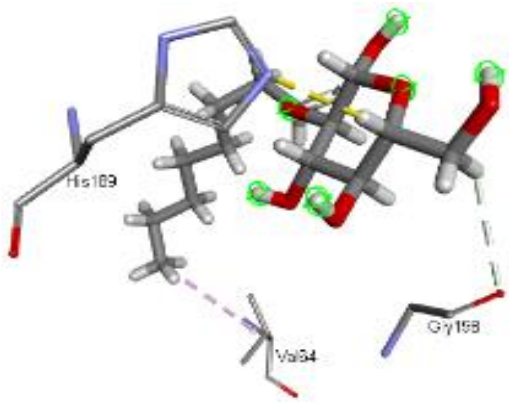

Figure 19: 3D image of interacting residues of 5FGN receptor and AMP (KPQAVFPWWW)

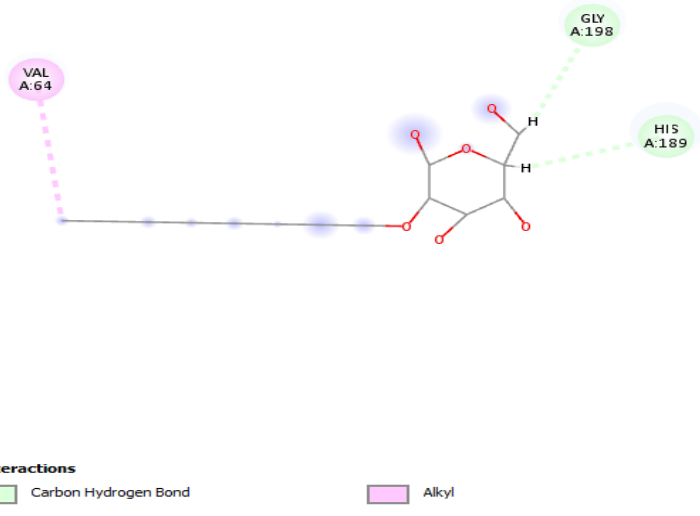

Figure 20: 2D image of interacting residues of 5FGN receptor and AMP (KPQAVFPWWW)

SUMMARY

V SUMMARY

Colibacillosis and Salmonellosis are the most common bacterial diseases in poultry. Antimicrobial drug resistance is a major concern in current bacterial diseases and AMP could serve a better alternate to overcome it. we screened 10 AMPs with appropriate antibacterial activity based on the physiochemical properties of antimicrobial peptides like length (≤10 in number), charge (+1 to +5) and hydrophobic residue (41% to 50%) from the Antimicrobial Peptide Database (APD3). The bacterial targets of *E coli* and *Salmonella* are 3ITA which is a penicillin binding protein (PBP 6). few studies on PBP6 and 5FGN which is the 3D structure of LptA (PDB ID: 5FGN; Neisseria meningitidis), the best template of MCR-1 of *Salmonella* SAUVM isolates that their functions are necessary for the health of the organism, especially for maintaining normal cell morphology.

For docking study, 3ITA and 5FGN receptor is prepared and the grid box was generated with the active site information of both receptors and performed docking with screened 10 AMPs by using AutoDock Vina 1.1.2 version. It generated the output with the docking score or binding affinity (Kcal/mol). Among them, AP00551 found to be best with −8.7 Kcal/mol at 3ITA penicillin-binding protein of *E. coli* and AP01357 found to be best with −9.4 Kcal/mol at lipid A phosphoethanolamine transferase for Salmonellosis. Residues involved in interaction between 3ITA and AP00551 are TRP 4, HIS 5, ARG 6, SER 40, ARG 194, THR 212 and ARG 244 obtained by using BIOVIA® Discovery Studio. Residues involved in interaction between 5FGN and AP01357 are VAL 64, HIS 189 and GLY 198.

Modification of the AMPs are done by substituting phenylalanine with hydrophilic amino acids and are docked with bacterial targets 3ITA and 5FGN. It generated the output with the docking score or binding affinity (Kcal/mol). Among them, sequence IPIAVFP found to be best with −7.0 Kcal/mol at 3ITA, penicillin-binding protein of *E. coli* and the sequence FIWWII found to be best with −8.7 Kcal/mol at lipid A phosphoethanolamine transferase for Salmonellosis. Residues involved in interaction between 3ITA and IPIAVFP are ALA 4, VAL 5, PHE 6, PRO 7, SER 40, ARG 194, THR 212 and ARG 244

obtained by using BIOVIA® Discovery Studio. Residues involved in interaction between 5FGN and FIWWII are VAL 64, HIS 189 and GLY 198.

AMPs are modified by end-tagging of tryptophan i.e., WWW in the C-terminal of each screened AMPs from the APD3 database and are docked with bacterial targets 3ITA and 5FGN. It

generated the output with the docking score or binding affinity (Kcal/mol). Among them, sequence FFHLHFHYWWW found to be best with −8.6 Kcal/mol at 3ITA, penicillin-binding protein of *E. coli* and the sequence KPQAVFPWWW found to be best with −9.0 Kcal/mol with 5FGN lipid A phosphoethanolamine transferase for Salmonellosis. Residues involved in interaction between 3ITA and IPIAVFP are THY 8, TRP 9, TRP 10, SER 40, ARG 194, THR 212 and ARG 244. obtained by using BIOVIA® Discovery Studio. Residues involved in interaction between 5FGN and KPQAVFPWWW are VAL 64, HIS 189 and GLY 198 obtained by using BIOVIA® Discovery Studio and also generated 3D and 2D images.

From the docking studies with the screened AMPs and modified AMPs with the bacterial targets. For *E coli* we found AP00551 exhibits highest binding energy of -8.7 Kcal/mol and followed by the AMP sequence FFHLHFHYWWW shows of binding energy of -8.6 Kcal/mol. For *Salmonella* we found AP01357 shows highest binding energy of -9.4 Kcal/mol followed by the AMP sequence KPQAVFPWWW of binding energy of -9.0 Kcal/mol.

Overlapping binding residues of the AMPs AP00551, IPIAVFP sequence and FFHLHFHYWWW sequence with *E coli* target 3ITA are SER 40, ARG 194, THR 212, ARG 244. For *Salmonella* target of 5FGN with the AMPs AP00551, FIWWII sequence and KPQAVFPWWW sequence are VAL 64, HIS 189 and GLY 198.

Future outline of work:

With the screened AMP's we can perform docking for different bacterial targets of the poultry diseases and also for other diseases.

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VI REFRENCES

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