Review Article

Antibiotic-Peptide Conjugates as Potential Quorum Sensing Inhibitors in Biofilm-Producing MDR Pathogens: An Updated Review

Ayantika Samanta, Srestha Das, Shreya Banerjee, Samadrito Chatterjee, Tamalika Chakraborty*
Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F, Nilgunj Road, Sodepur Panihati, Kolkata-700114, India.
***Corresponding author's E-mail:** tamalika.chakraborty@gnipst.ac.in

Received: 03-01-2025; **Revised:** 28-04-2025; **Accepted:** 10-05-2025; **Published online:** 15-05-2025.

ABSTRACT

Bacteria have various strains some can be beneficial and some are pathogenic. The pathogenic strains of bacteria are dangerous as they have caused several critical and untreatable diseases and till now their pathogenesis is ever increasing. Those diseases are becoming incurable because of the increment of the resistant mechanism of bacteria. Traditional antibiotics are not effective against pathogenic strains of bacteria like MRSA, *E.coli, Streptococcus pneumonia; Klebsiella* sp. due to the evolution of drug resistance mechanisms, etc. *A* new strategy has been introduced which is combinatorial therapy. In this combinatorial therapy, antimicrobial peptides (AMP) and antibiotics are combined and applied against drug-resistant bacteria. Much research reveals that combinatorial therapy shows more efficacy against the pathogenic strain than the antibiotic or AMP alone. This review focuses specifically on the structure of AMP, how it works, and helps antibiotics to enhance their actions specifically as combinatorial therapy and its therapeutic output.

Keywords: Antibiotic resistance, Biofilm, Quorum sensing, antimicrobial peptide, combinatorial therapy.

INTRODUCTION

Antibiotics are drugs that are utilized to control bacterial disease. Antibiotics function either by killing the bacteria called bactericidal activity or by limiting their growth called bacteriostatic activity. Through these activities, antibiotics help our immune system to recognize foreign particles more specifically and combat against infection. Since their discovery Antibiotics have been effective against many bacterial infections.

But nowadays, antibiotic resistance has been increasing very rapidly due to improper use of antibiotics like incompletion of the course of antibiotics, excessive use of antibiotics, etc. Thus, in the present day, combinatorial therapy is preferable and this review aims to show some synergism between antibiotic and AMP that is the main cause of effective combinatorial therapy against pathogenic bacteria.

Table 1: Mechanism of action of antibiotics:

Mode of action	Targets	Drug class	Specific drugs example	References
Cell wall synthesis inhibition	Penicillin-binding protein	β-lactams	Penicillin G, amoxicillin, and cephalosporin C	1
	Peptidoglycan subunits	Glycopeptides	Vancomycin	2
Inhibition of protein synthesis	30 s subunit	Aminoglycosides and tetracyclines	Streptomycin, gentamicin, neomycin, tetracycline, and doxycycline	3
	50 s subunit	Macrolides, chloramphenicol, and oxazolidinones	Erythromycin, azithromycin, chloramphenicol, and linezolid	3
Inhibition of nucleic acid synthesis	RNA	Rifamycin	Rifampin	4
	DNA	Fluoroquinolones	Ciprofloxacin and ofloxacin	5
Anti-metabolites	Folic acid synthesis enzymes	Sulfonamides and trimethoprim	Sulfamethoxazole, dapsone, and trimethoprim.	6
Disrupt membranes	Lipopolysaccharides	Polymyxins	Polymyxin B and colistin	7

Mechanisms of antibiotic resistance:

Bacteria become resistant to antibiotics by different mechanisms. Four mechanisms of resistance have been identified, each of which involves alternation of a different microbial structure.

1. **Development of enzyme:** Some microorganisms release some enzyme that specifically targets the antibiotics or modifies the structure of the drug, hence becoming resistant.

   For example – Bacteria that synthesize the enzyme Penicillinase are resistant to the bactericidal effect of penicillin, as it breaks the beta-lactam ring of penicillin and interferes with its mechanism.[8]

2. **Alternation of target:** An antimicrobial drug generally recognizes and binds to a specific receptor molecule in a bacterium and prevents its growth or kills it. Minor Structural changes in the target which result from mutation prevent the drug from binding to the target.[9]

   For example- a change in rRNA, the target for the erythromycin, prevents these drugs from interfering with ribosome function. This mechanism allows a formerly inhibited reaction to occur.

3. **Alternation of an enzyme:** This mechanism allows a formerly inhibited reaction to occur.[9]

   For example- this mechanism is found among certain sulfonamide resistance bacteria. These organisms have developed an enzyme that has a very high affinity for para-aminobenzoic acid and a very low affinity for sulfonamide. Consequently, even in the presence of sulphonamide, the enzyme works well to allow the bacterial function.

4. **Alternation of metabolic pathway:** This mechanism bypasses a reaction inhibited by an antimicrobial agent that occurs in certain sulfa drug resistance in bacteria[10]. These organisms have acquired the ability to use readymade folic acid from the environment and no longer need to make it from para-aminobenzoic acid.

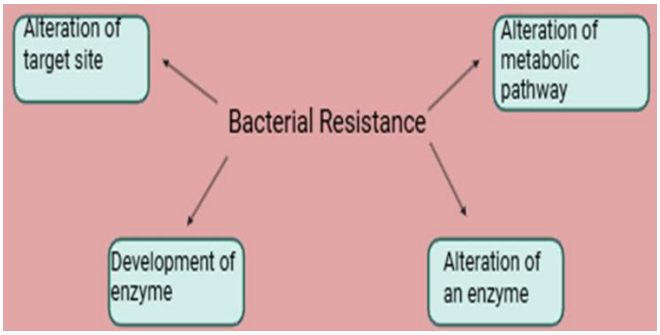

Figure 1: Mechanism of bacterial resistance

Quorum sensing in the mechanism of biofilm formation:

Biofilm is a collection of microorganisms enclosed in a self-produced matrix of EPS (extracellular polymeric substances) that typically comprises polysaccharides, proteins, lipids, and nucleic acids. Naturally, biofilm is the aggregation of bacteria. By forming Biofilm bacteria protect themselves against any type of drugs. Bacteria weaken the immune system of the host and they remain within the self-produced matrix and thus can inhibit the effect of antibiotics. Bacteria exchange different signals by producing biofilm and remain alive for a very long time. Biofilm forms on the surface of medical devices like lenses, catheters, and cardiac pacemakers [11].

Extracellular polymeric substance significantly contributes to the development of biofilms which is augmented by environmental factors. The EPS matrix consists of protein, nucleic acid, e-DNA, and glycoprotein[12]. Bacteria appear to activate certain genes for polysaccharide synthesis only after a stable attachment to a substrate has occurred, according to molecular studies. Biofilms can develop on any nutrient-rich substance, but smooth surfaces are preferable.

The role of biofilms in antimicrobial resistance (AMR) is very complex and can have a big impact on resistance. Bacteria growing in a biofilm can show 10 to 1,000 times greater antibiotic resistance compared to the same bacteria growing as planktonic cells (free-floating bacteria)[13].

In bacteria, frequent mechanisms of antibiotic resistance comprise point mutations, enzymes, and efflux pumps. Nonetheless, it is improbable that these mechanisms account for the resistance observed in biofilm organisms. Different elements collaborate within a biofilm to promote more resistance in bacteria against antibiotics.

In particular, 3 mechanisms for antibiotic resistance of bacteria in biofilms are important:

1. **Resistance at the Biofilm Surface:** The first mechanism involves antibiotics struggling to penetrate biofilm surfaces mainly the EPS matrix due to its complex structure, leading to faster deactivation at the surface, though this varies among different biofilms[14].

2. **Resistance Within Biofilm Microenvironments:** Planktonic bacteria respond to environmental fluctuation so rapidly. Antibiotics penetrating biofilm face challenging conditions, including reduced oxygen, accumulated waste, and pH variations, affecting their efficacy differently based on their structure and mechanism of action.

   For example, *S. epidermidis* forms a biofilm and becomes more resistant to antibiotics in an acidic environment[15]

3. **Resistance of Bacterial "Persister" Cells:** Inside biofilms, bacteria can enter a dormant "spore-like" state, known as persister cells, which are highly antibiotic tolerant. Persister cells show temporary resistance and this resistance is due to their metabolic inactivity and dormancy. Persister cells were observed in *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans*, *A.*

baumannii, and *B. cereus.* They revert to their original susceptibility once released or begin to divide again[16].

MECHANISM OF QUORUM SENSING FORMATION:

Quorum sensing is a process by which bacteria establish connections between them. Different signal molecules mainly help bacteria to form biofilm. Three signal molecules Acyl homoserine lactone(AHL), Oligopeptide, and Furan borate di-ester are the reason for biofilm formation in Gram-negative and Gram-positive and both bacteria[17].Small oligopeptide represents an autoinducing signal molecule, used as a signal molecule for quorum sensing in Gram-positive bacteria. By modifying the leader autoinducing peptide (AIP) precursors molecules are formed. When the cell density increases, bacteria synthesize a large number of virulence factors and that's why pathogenicity increases. Oligopeptides made this process as a response to regulate gene expression and stimulate cells[17].

When signal molecule oligopeptide is secreted to certain concentrations, it will bind to the receptor protein on the cell membrane and regulate the gene expression by activating or inhibiting the gene of interest. Acyl homoserine lactone (AHL) represents another signal molecule. Autoinducer 1 represents AHL, present in Gram-negative bacteria, and can diffuse freely into and out of bacterial cells. AHL is a synthetic product in the LuxR -LuxI system of gram-negative bacteria [17].LuxR binding protein is a transcriptional activator, encoded by LuxR.When acyl homoserine lactone becomes active then it regulates that transcriptional activator. LuxI encodes LuxI protein, a type of AHL synthetase. After binding of AHL and LuxR, dimerization or multimerization occurs [17].The multimerization product activates or inhibits the expression of the target gene by binding to the upstream regulatory region of the target gene.

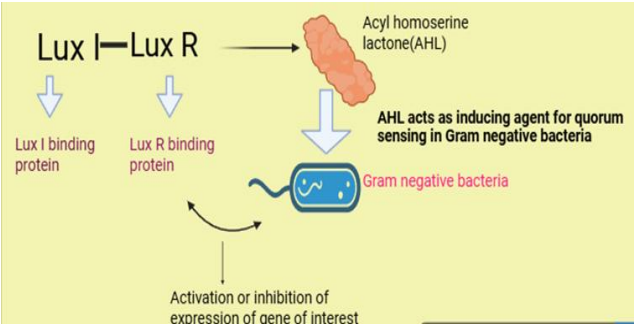

Figure 2: Acyl homoserine lactone acts as an Autoinducing agent to establish quorum sensing

Another signal molecule represented by autoinducer 2(AI 2) is Furan borate diester. AI 2 differs from AI 1 in the phenomenon that AI 2 mediates the interspecies quorum sensing system that is bacteria regulate gene expression by receiving signals from AI 2, released by foreign bacteria[17].

Antibiotic -peptide conjugate-an emerging strategy:

Antibiotic-peptide conjugate is a hybrid molecule that is made by combining antimicrobial peptide (AMP) and antibiotics. Antibiotic-peptide conjugate (APC) is the modern strategy to combat against biofilm formation. By combining AMP with antibiotics, antibiotics enhance the activity of AMP.APC sometimes helps to overcome the problem of antimicrobial resistance.

The resistance mechanism of bacteria is increasing day by day. Bacteria develop resistance by combining various processes like efflux pump activation, Biofilm formation, and Synthesis of particular proteins that can protect the target site. AMP specifically targets microbial membranes because their membrane is made up of lipopolysaccharide (LPS), lipoteichoic acid, etc. but the mammalian membrane is made up of zwitterionic phospholipids, sphingomyelin and cholesterol. As AMP itself is a cationic molecule it always binds to the anionic molecules. Thus, AMP targets specifically microbial membranes[18].

AMP has various diversity it is classified into four groups based on their structure-Alpha helical, Beta sheet, Extended, and Loop peptides. Mostly all AMPs are amphipathic. AMPs first adopt a secondary structure when they come in contact with the membrane of pathogens then they bind to the cell membrane by electrostatic interaction and increase the cell permeability, finally they disrupt the cell membrane [18].But except these mechanisms AMP also inhibits the quorum sensing process by direct way or indirect way.

As mentioned above AMP first formed a secondary structure in contact with the membrane of the cell, various models of AMP were observed like the toroidal pore model, Barrel stave model, and Carpet-like model.

In the Toroidal pore model, AMPs are present on the surface of the membrane and continuously induce the membrane so that the membrane can pass through the pores and form a pore built up by those peptides[18]. In the Barrel stave model the attached peptides are assembled on the outer membrane and as a result are inserted into the cell membrane[18].In the Carpet like the model, Pores are not formed like previous two models. Peptides are gathered in a parallel way to the bacterial membrane, covering the whole membrane like a carpet[18]. Then, those attached peptides instigate permeabilization and membrane disruption and lastly, micelles are formed.

It was observed that combinatorial therapies are more effective rather than monotherapies. In combinatorial therapy two types of antimicrobial agents are used, as a result, Bacteria cannot become resistant rapidly against those agents and the first compound will decrease the resistance against the second compound and vice versa. In combinatorial therapy, the dose of the drugs is used in minimum quantity and it is enough effective against those pathogenic bacteria.

Importance of combinatorial therapy in the development of advanced therapeutics in drug-resistant pathogen.

Drug resistance among bacteria is increasing day by day. The conventional antibiotics are not very effective against that MDR pathogen. So, it was observed by various experiments

that some AMPs show antibiofilm activity. When those antibiotics are combined with some conventional antibiotics, they most of the time show a synergistic effect and this synergism shows more effective antibiofilm activity rather than those antibiotics alone or those AMP alone. Also, AMP enhances the activity of antibiotics to eradicate the biofilm-forming bacteria, disrupt cell membranes, interfere with nucleic acid synthesis, etc.

Most of the time bacteria do not become resistant against combinatorial therapy because in this type of therapy the conjugate targets many sites of bacteria and it is very difficult for bacteria to develop resistance very rapidly for those multiple sites. Thus, at this time combinatorial therapy is preferable to combat biofilm-related infections.

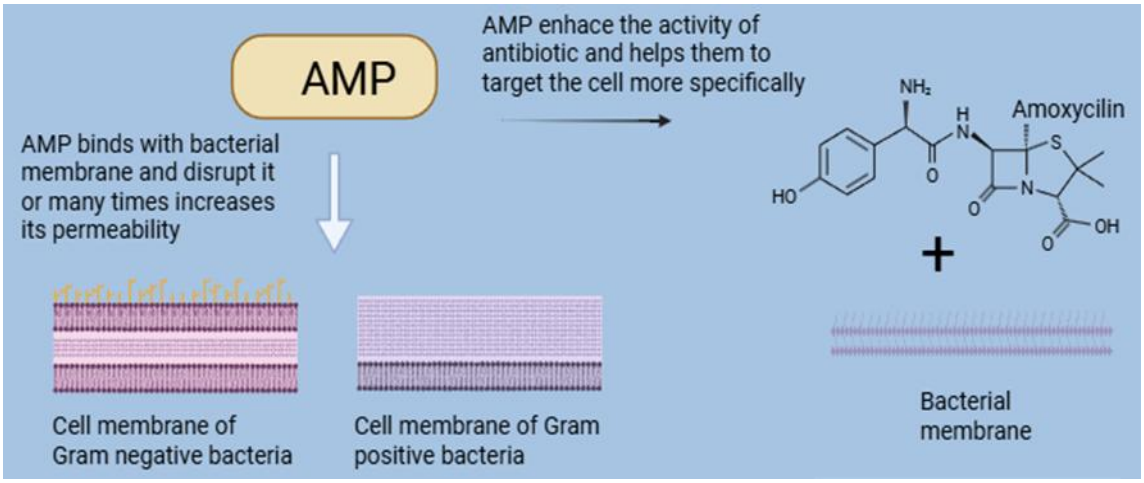

Figure 3: Mechanism of action of AMP

Innovative combination of Antimicrobial peptide and antibiotics, showing synergism against biofilm formation:

Ciprofloxacine-Melimine or Mel 4 conjugate: Ciprofloxacin is a broad-spectrum antibiotic that is mainly effective against both Gram-positive and Gram-negative bacteria. Ciprofloxacin naturally binds to DNA gyrase and Topoisomerase IV and causes conformational changes in DNA. Then DNA replication stops. But some bacteria including *Staphylococcus aureus* became resistant to this antibiotic.

Melimine and Mel 4 are cationic peptides. It was observed that those peptides have antibiofilm activity. It was also examined that ciprofloxacin alone cannot prevent the growth of the biofilm-forming bacteria rather when it is combined with Melimine or Mel 4 it shows better efficacy against biofilm formation[19].

Caprine bactenecinChBac 3.4-Oxacillin or Ofloxacin conjugate: Caprine bactenecin is an AMP derived from the leukocyte of the domestic goat *Capra hircus.* This AMP is effective against both Gram-positive and Gram-negative bacteria, this is a linear AMP and it has dual activity in low concentration it binds to the aminoacyl site of bacterial ribosome and disrupts

the protein folding process. In higher concentrations, it increases the permeability of the bacterial cell membrane[20].t was observed that when this AMP ChBac 3.4 (1-14)-NH2 combines with Oxacillin or Ofloxacin it shows high efficacy against biofilm formation and much more effective against MDR pathogens like *E.coli, Staphylococcus aureus* etc[20].

Tobramycin-IDR1018 conjugate: Tobramycin is a conventional antibiotic that has broad-spectrum antimicrobial activity. This anti-biotic mainly targets bacterial ribosomes and disrupts the protein formation process.

IDR 1018 is a type of AMP that shows great activity against nosocomial infection-causing bacteria like *P. aeruginosa*, *Escherichia coli*, *A. baumannii*, *K. pneumoniae*, methicillin-resistant *S. aureus*, *Salmonella enterica* serovar Typhimurium, and *Burkholderia cenocepacia.* The combinatorial therapy of Tobramycin and IDR 1018 shows a large decrement in bacterial MIC and even in a short time they can destroy the biofilm[21].

Melittin-Colistin conjugate: Colistin is an antibiotic that inhibits bacterial growth. It has a wide range of antimicrobial activity.

Melittin, an AMP shows antibiofilm activity against biofilm-forming bacteria by disrupting their cell membrane, by down-regulation the gene cause for biofilm formation.

The synergism between Melittin and colistin shows higher antibiofilm activity against *Acinetobacter baumannii* [22]. The synergism was effective against biofilm formation by disrupting the integrity of bacterial cell membranes, RNA polymerase, DNA gyrase, etc[22].

Table 2: Antibiotic conjugate AMPs with their mechanism of action

Conjugate name	Antibiotic	AMP/ Peptides	Target Pathogens	Mechanism	References
Vancapticins	Vancomycin	Polysines with MIE	MRSA, Gram-positive resistant strains	Dual action, improved drug accumulation, and membrane insertion	[23]
Vancomycin-Ahx-r8	Vancomycin	D-octaarginin (r8)	MRSA, *Enterococcus faecium* (VRE), *Staphylococcus aureus* (VISA)	Intracellular access, membrane penetration, and suppression of cell wall synthesis	[24]
FU002	Vancomycin	Hexaarginin (Cys-tagged)	*Enterococcus faecium* (VRE), *Staphylococcus aureus* (VRSA)	Superior pharmacokinetics and dual targeting: D-Ala-D-Ala + membrane disruption	[25]
Van-Hec	Vancomycin	Hectate (Hec)	MRSA, *Staphylococcus aureus* (VRSA)	Bacterial wall integrity disruption and synergistic death through various mechanisms	[26, 27]
VPCs (e.g. VPC11)	Vancomycin	LPS-binding peptides	*E. coli AB1157* *A. baumannii,* *K. pneumoniae* *PA01* *P. aeruginosa*	LPS interaction, gram-negative targeting, and altered antimicrobial profile	[28,29]
Cephalotin-D-Bac8c (Leu2,5)	Cephalotin	D-Bac8c (Leu2,5)	*E. coli*, MRSA	Prodrug: decreased toxicity due to β-lactamase-induced AMP release	[30]
MSI-78-ACA/ADCA	Cephalosporin	MSI-G78, CA (1-7) M (2-9), des-Chex1	*A.baumannii*, MDR, *A.baumannii156*	Direct membrane binding combined with intracellular β-lactam activity	[31]
Amp-2P2-2, Amp-Oncocin (e.g. 37)	Ampicillin	Magainin analog 2P2-2/Oncocin	*A. baumannii ATCC19606,* *E. coli BW25113,* *S.epidermidis ATCC12228*	AMP facilitates delivery; β-lactam activity is restored in resistant bugs; disulfide-cleavable linker	[32, 33]
Pentobra	Tobramycin	12-merAMP	*P.acnes, E. coli,* *S.aureus*persisters	Ribosomal suppression within cells combined with membrane disruption	[34, 35, 36]

Application of AI in the design of Antimicrobial peptide:

Artificial Intelligence(AI)is now the solution to every problem. Even AI-driven AMP synthesis is happening. It was proved that AI-driven AMP is more effective than normal AMP in combinatorial therapy.

Recently, Machine Learning (ML) algorithms have been used for the detection of the exact synergism mechanism between AMP and other antimicrobial compounds. These methods reduce the time most importantly and give accurate information[37].

By using AI AMP of our choice can be structured. We can design the peptide by categorizing their properties and can design AMP by selecting their properties like their toxicity, and their activity of biofilm disruption, and by this process Novel AMP can be achieved.

Challenges and limitations:

Many of the appealing characteristics of a novel antibiotic class are present in AMPs, including their wide range of activity, low rate of bacterial resistance, and unique mode of action that involves the development of cytoplasmic membrane pores[38]. AMPs often show excellent stability across a broad pH and temperature range, which could be advantageous for larger manufacturing and formulation into deliverable goods. Because of their unique mechanism of action, AMPs are also less harmful to eukaryotic cells, opening a broad therapeutic window. There have also been reports of low concentrations of AMPs that have shown disruptive and inhibitory qualities that eradicate even well-established biofilms. Additionally, AMPs neutralize endotoxins, work in animal models, and exhibit synergy with traditional antibiotics [39]. Because AMPs are drawn to the negatively charged lipid bilayer structure of bacterial

membranes, resistance to them is comparatively uncommon [39]. The most common method of action for AMPs is permeabilization and the creation of pores within the cytoplasmic membranes, which allows them to affect bacteria that grow slowly or not at all [40]. The capacity to function at several phases of biofilm development and by various modes of action, such as down-regulating QS, eliminating pre-formed biofilm, preventing biofilm formation, and preventing adhesion, are additional potentials of AMPs [41]. Additionally, AMPs frequently work against bacterial strains that are resistant to many drugs. Although the functions of QS in biofilm formation are well understood, there are still few reports of AMPs that can block the quorum sensing systems employed by the various bacterial pathogens.

Even though AMPs have promising properties as potential therapeutics, there are limitations that must be addressed for further development. The high concentrations of salt, anionic proteins, and polysaccharides found in biological fluids, and the inactivation of AMPs by host and bacterial proteases during infection cause AMPs to significantly lose their antimicrobial potency when present in biological fluids (such as serum and saliva) as opposed to non-physiological conditions (such as phosphate buffer)[42]. By forming a hydrated and charged environment around the bacterial surface, biofilms including bacterial DNA and other polymers can block cationic AMP LL-37's access [43]. Human cells could be harmed by AMPs that are not of human origin. The main ingredient in the venom of European honeybees (Apis mellifera) is melittin, an alkaline polypeptide with 26 amino acid residues that have been shown to be lytic to normal, healthy cells, including erythrocytes [44]. Low viscosity and difficulty administering high-dose protein formulations subcutaneously with a volume limitation of less than 1.5 mL are caused by poor physical-chemical characteristics, such as protein aggregation, particulate formation, and reversible self-association [45]. Additionally, AMPs may be vulnerable to proteolytic breakdown. Because of the intricate procedures required for their extraction, isolation, and purification, AMPs are costly and challenging to produce in high amounts[46] .

Future perspective and clinical trial:

Conventional antibiotics are anchored to an AMP or CPP using an appropriate bifunctional linker to create antibiotic-AMP–AMP conjugates. The N-terminus and the C-terminus are the two places of attachment for the peptide. Generally speaking, the linker falls into one of two categories: cleavable stimuli-responsive linker or stable covalent linker. Upon entering the bacterial cell, the antibiotic and the AMP could each work separately, targeting their respective locations, thanks to cleavable stimuli-responsive linkers. On the other hand, the conjugate molecules work as a single, multimodal antibacterial chemical if they stay together. As a result, they can attach to and influence their targets at the same time, possibly exhibiting different dynamics from the constituent parts.

AMPs may be able to inhibit the expansion of biofilms while not always eliminating all microorganisms, such as Nal-P-113 against *Porphyromonas gingivalis* W83 biofilm formation; thus, the authors propose combining it with other drugs currently used for the oral treatment of this potentially virulent bacterium. Similarly, some studies report that the inclusion or structural modification of AMP could improve their synergistic or combined effect; for example, chimeric peptide-Titanium conjugate (TiBP1-spacer-AMP y TiBP2-spacer-AMP) against *Streptococcus mutans, Staphylococcus epidermidis, and Escherichia coli*[47] , A3-APO (proline-rich AMP) combined with imipenem against ESKAPE pathogens, biofilm-forming bacteria, and in vivo murine model [48,49]. Furthermore, it was noted that adding fatty acids to the C terminal could increase the specificity and efficacy of AMPs against superbugs and their corresponding biofilms [50].

By using AI, various types of AMP can be synthesized. We can design peptides of our own choice. By knowing the exact effect of AMPs and their target sites, different AI tools can form those AMPs that show more efficacy against biofilm rather than normal. In the future experiments should be done about how AI can make the delivery system of those Antibiotic peptide conjugates more preciously so that mammalian cells remain unaffected.

Table 3: Strategies to enhance the efficacy of antibiotic peptide conjugate

Strategies	Description	Examples with benefits	References
Prodrug	In order to lessen toxicity, an inactive form of AMP becomes active in infection settings.	P-dpMtx: Targets Mycobacterium tuberculosis in macrophages by combining an anionic peptide, cephalosporin linker, and delivery peptide. Pro-WMR: Less harmful prodrug reactivated in lung fluid from cystic fibrosis by neutrophil elastase	[51], [52], [53]
Conjugation	AMPs can be covalently attached to other functional molecules to improve stability or targeting.	Increased activity in high salinity is a result of AMP rather than the antibiotic Levofloxacin–Pep-4. Conjugate of dithiocarbazate and CPP: Wide-ranging effect on *S. aureus*. Hyperbranched polyglycerol plus aurein 2.2: Enhanced effectiveness and biocompatibility	[54], [55], [56]

Combined use with antibiotics	Co-administration of conventional medications to improve efficacy and lower resistance.	Plectasin + Teicoplanin: Effective against VRE (*E. faecalis*) Colistin + Fusidic Acid: A powerful combination against multidrug-resistant *A. baumannii* Polymyxin B + Rifampicin/Carbapenem: Prevents resistance in *A. baumannii*	[57,58,59,60,61,62]
Induction in host cells	Using outside cues to initiate the host's natural AMP expression	Butyrate and vitamin D work together to promote the expression of LL-37. UV/Sunlight Therapy: Helps treat tuberculosis by stimulating the generation of LL-37 in the skin and lungs. Aroylated Phenylenediamines: A New Class of Inducing Peptides	[63, 64]

The majority of AMPs currently undergoing preclinical and clinical studies were created with topical uses in mind. Catheter site infections, cystic fibrosis, acne, and wound healing are a few examples of indications. Despite showing promise in Phase III clinical trials, two AMPs, Omiganan and Pexiganan, have not been approved for clinical use. Pexiganan, a synthetic variant of magainin 2 and the most studied AMP in terms of drug development, was developed as a novel topical broad-spectrum antibiotic to treat mild-to-moderate diabetic foot ulcer infections [65, 66], and Omiganan was developed as a topical gel to prevent catheter-associated infections. New antimicrobial peptides with enhanced kinetics, selectivity, and killing effectiveness against specific bacteria are called Selectively Targeted Antimicrobial Peptides (STAMPs). The semi-synthetic lipoglycopeptide oritavancin is being developed therapeutically to treat significant Gram-positive infections, including vancomycin-resistant *S. aureus* (VRSA), methicillin-susceptible [MSSA], and methicillin-resistant [MRSA]. Vancomycin's killing kinetics are much slower than its [67]. In clinical settings, a class of CAMPs called polymixins has been used as a last option to treat Gram-negative bacterial infections [68].

CONCLUSION

The combinatorial therapy is giving promising results. In the future work should be done on the delivery system of this combination (antibiotic-peptide conjugate). Through AI different unique AMPs will be generated and those also definitely give better results. This combinatorial therapy can be used to treat many untreatable diseases but overuse or improper use may lead to resistance against them like antibiotic resistance. Thus, this therapy should be done in a dose-dependent manner.

In the near future, many more AMPs will be introduced in the field of medical science, and hope those AMPs will show great efficacy in combination with Antibiotics against drug-resistant pathogens.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Schneider T, Sahl HG. An oldie but a goodie–cell wall biosynthesis as antibiotic target pathway. International Journal of Medical Microbiology. 2010 Feb 1;300(2-3):161-9.DOI: https://doi.org/10.1016/j.ijmm.2009.10.005
2. Sarkar P, Yarlagadda V, Ghosh C, Haldar J. A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. Medchemcomm. 2017;8(3):516-33. DOI:10.1039/c6md00585c
3. McCoy LS, Xie Y, Tor Y. Antibiotics that target protein synthesis. Wiley Interdisciplinary Reviews: RNA. 2011 Mar;2(2):209-32.DOI: https://doi.org/10.1002/wrna.60
4. McClure WR, Cech CL. On the mechanism of rifampicin inhibition of RNA synthesis. Journal of Biological Chemistry. 1978 Dec 25;253(24):8949-56. DOI:10.1016/s0021-9258(17)34269-2
5. Hooper DC. Mechanisms of action of antimicrobials: focus on fluoroquinolones. Clinical infectious diseases.2001Mar15;32(Supplement_1):S9-15.
6. Capasso C, Supuran CT. Sulfa and trimethoprim-like drugs–antimetabolites acting as carbonic anhydrase, dihydropteroate synthase and dihydrofolate reductase inhibitors. Journal of enzyme inhibition and medicinal chemistry. 2014 Jun 1;29(3):379-87.DOI: https://doi.org/10.3109/14756366.2013.787422
7. Ayoub Moubareck C. Polymyxins and bacterial membranes: a review of antibacterial activity and mechanisms of resistance. Membranes. 2020 Aug 8;10(8):181.DOI:10.3390/membranes10080181
8. Uddin TM, Chakraborty AJ, Khusro A, Zidan BR, Mitra S, Emran TB, Dhama K, Ripon MK, Gajdács M, Sahibzada MU, Hossain MJ. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of infection and public health. 2021 Dec 1;14(12):1750-66. DOI:10.1016/j.jiph.2021.10.020
9. Cag Y, Caskurlu H, Fan Y, Cao B, Vahaboglu H. Resistance mechanisms. Annals of translational medicine. 2016 Sep;4(17):326.DOI: 10.21037/atm.2016.09.14

10. Giedraitienė A, Vitkauskienė A, Naginienė R, Pavilonis A. Antibiotic resistance mechanisms of clinically important bacteria. Medicina. 2011 Mar;47(3):19.DOI: https://doi.org/10.3390/medicina47030019
11. Asma ST, Imre K, Morar A, Herman V, Acaroz U, Mukhtar H, Arslan-Acaroz D, Shah SR, Gerlach R. An overview of biofilm formation–combating strategies and mechanisms of action of antibiofilm agents. Life. 2022 Jul 23;12(8):1110. DOI:10.3390/life12081110
12. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules. 2009 Jul 13;14(7):2535-54. DOI:10.3390/molecules14072535
13. Devanga Ragupathi NK, Veeraraghavan B, Karunakaran E, Monk PN. Biofilm-mediated nosocomial infections and its association with antimicrobial resistance: Detection, prevention, and management. Frontiers in Medicine. 2022 Aug 1;9:987011.DOI: https://doi.org/10.3389/fmed.2022.98701
14. Venkatesan N, Perumal G, Doble M. Bacterial resistance in biofilm-associated bacteria. Future microbiology. 2015 Nov 1;10(11):1743-50. DOI:10.2217/fmb.15.69
15. Behbahani SB, Kiridena SD, Wijayaratna UN, Taylor C, Anker JN, Tzeng TR. pH variation in medical implant biofilms: Causes, measurements, and its implications for antibiotic resistance. Frontiers in Microbiology. 2022 Oct 31;13:1028560. DOI:10.3389/fmicb.2022.1028560
16. Almatroudi A. Biofilm resilience: Molecular mechanisms driving antibiotic resistance in clinical contexts. Biology. 2025 Feb 6;14(2):165. DOI:10.3390/biology14020165
17. Zhao X, Yu Z, Ding T. Quorum-sensing regulation of antimicrobial resistance in bacteria. Microorganisms. 2020 Mar 17;8(3):425. DOI:10.3390/microorganisms8030425
18. Reinhardt A, Neundorf I. Design and application of antimicrobial peptide conjugates. International journal of molecular sciences. 2016 May 11;17(5):701. DOI:10.3390/ijms17050701
19. Yasir M, Dutta D, Willcox MD. Enhancement of antibiofilm activity of ciprofloxacin against Staphylococcus aureus by administration of antimicrobial peptides. Antibiotics. 2021 Sep 24;10(10):1159. DOI:10.3390/antibiotics10101159
20. Zharkova MS, Orlov DS, Golubeva OY, Chakchir OB, Eliseev IE, Grinchuk TM, Shamova OV. Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics—a novel way to combat antibiotic resistance?. Frontiers in cellular and infection microbiology. 2019 Apr 30;9:128. .DOI:10.3389/fcimb.2019.00128
21. Pletzer D, Hancock RE. Antibiofilm peptides: potential as broad-spectrum agents. Journal of bacteriology. 2016 Oct 1;198(19):2572-8. DOI:10.1128/JB.00017-16
22. Mirzaei R, Esmaeili Gouvarchin Ghaleh H, Ranjbar R. Antibiofilm effect of melittin alone and in combination with conventional antibiotics toward strong biofilm of MDR-MRSA and-Pseudomonas aeruginosa. Frontiers in Microbiology. 2023 Feb 20;14:1030401. DOI:10.3389/fmicb.2023.1030401
23. Kahne D, Leimkuhler C, Lu W, Walsh C. Glycopeptide and lipoglycopeptide antibiotics. Chemical reviews. 2005 Feb 9;105(2):425-48. DOI:10.1021/cr030103a
24. Blaskovich MA, Hansford KA, Gong Y, Butler MS, Muldoon C, Huang JX, Ramu S, Silva AB, Cheng M, Kavanagh AM, Ziora Z. Protein-inspired antibiotics active against vancomycin-and daptomycin-resistant bacteria. Nature communications. 2018 Jan 2;9(1):22. DOI:10.1038/s41467-017-02123-w
25. Umstätter F, Domhan C, Hertlein T, Ohlsen K, Mühlberg E, Kleist C, Zimmermann S, Beijer B, Klika KD, Haberkorn U, Mier W. Vancomycin resistance is overcome by conjugation of polycationic peptides. Angewandte Chemie International Edition. 2020 Jun 2;59(23):8823-7. DOI:10.1002/anie.202002727
26. Rivero-Müller A, Vuorenoja S, Tuominen M, Wacławik A, Brokken LJ, Ziecik AJ, Huhtaniemi I, Rahman NA. Use of hecate–chorionic gonadotropin β conjugate in therapy of lutenizing hormone receptor expressing gonadal somatic cell tumors. Molecular and cellular endocrinology. 2007 Apr 15;269(1-2):17-25. DOI:10.1016/j.mce.2006.11.016
27. Jelinkova P, Splichal Z, Jimenez AM, Haddad Y, Mazumdar A, Sur VP, Milosavljevic V, Kopel P, Buchtelova H, Guran R, Zitka O. Novel vancomycin-peptide conjugate as potent antibacterial agent against vancomycin-resistant Staphylococcus aureus. Infection and drug resistance. 2018 Oct 10:1807-17. DOI:10.2147/IDR.S160975
28. Meredith TC, Aggarwal P, Mamat U, Lindner B, Woodard RW. Redefining the requisite lipopolysaccharide structure in Escherichia coli. ACS chemical biology. 2006;1(1):33–42.DOI:10.1021/cb0500015
29. Shi W, Chen F, Zou X, Jiao S, Wang S, Hu Y, Lan L, Tang F, Huang W. Design, synthesis, and antibacterial evaluation of vancomycin-LPS binding peptide conjugates. Bioorganic & Medicinal Chemistry Letters. 2021 Aug 1;45:128122. DOI:10.1016/j.bmcl.2021.128122
30. Desgranges S, Ruddle CC, Burke LP, McFadden TM, O'Brien JE, Fitzgerald-Hughes D, Humphreys H, Smyth TP, Devocelle M. β-Lactam-host defence peptide conjugates as antibiotic prodrug candidates targeting resistant bacteria. Rsc Advances. 2012;2(6):2480-92. DOI:10.1039/c2ra01351g
31. Li W, O'Brien-Simpson NM, Holden JA, Otvos L, Reynolds EC, Separovic F, Hossain MA, Wade JD. Covalent conjugation of cationic antimicrobial peptides with a β-lactam antibiotic core. Peptide Science. 2018 May;110(3):e24059. DOI:10.1002/pep2.24059
32. Azuma E, Choda N, Odaki M, Yano Y, Matsuzaki K. Improvement of therapeutic index by the combination of enhanced peptide cationicity and proline introduction. ACS Infectious Diseases. 2020 Aug 6;6(8):2271-8. DOI:10.1021/acsinfecdis.0c00387
33. Knappe D, Piantavigna S, Hansen A, Mechler A, Binas A, Nolte O, Martin LL, Hoffmann R. Oncocin (VDKPPYLPRPRPPRRIYNR-NH2): a novel antibacterial peptide optimized against gram-negative human pathogens. Journal of medicinal chemistry. 2010 Jul 22;53(14):5240-7. .DOI:10.1021/jm100378b
34. Williams HC, Dellavalle RP, Garner S. Acne vulgaris. The lancet. 2012 Jan 28;379(9813):361-72. DOI: 10.1016/s0140-6736(11)60321-8

35. Schmidt NW, Deshayes S, Hawker S, Blacker A, Kasko AM, Wong GC. Engineering persister-specific antibiotics with synergistic antimicrobial functions. ACS nano. 2014 Sep 23;8(9):8786-93.DOI: https://doi.org/10.1021/nn502201a

36. Schmidt NW, Agak GW, Deshayes S, Yu Y, Blacker A, Champer J, Xian W, Kasko AM, Kim J, Wong GC. Pentobra: a potent antibiotic with multiple layers of selective antimicrobial mechanisms against Propionibacterium acnes. Journal of Investigative Dermatology. 2015 Jun 1;135(6):1581-9.DOI: https://doi.org/10.1038/jid.2015.40

37. Olcay B, Ozdemir GD, Ozdemir MA, Ercan UK, Guren O, Karaman O. Prediction of the synergistic effect of antimicrobial peptides and antimicrobial agents via supervised machine learning. BMC Biomedical Engineering. 2024 Jan 17;6(1):1. DOI:10.1186/s42490-024-00075-z.

38. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nature biotechnology. 2006 Dec 1;24(12):1551-7. DOI:10.1038/nbt1267

39. Koczulla AR, Bals R. Antimicrobial peptides: current status and therapeutic potential. Drugs. 2003 Feb;63:389-406. DOI:

40. Batoni G, Maisetta G, Lisa Brancatisano F, Esin S, Campa M. Use of antimicrobial peptides against microbial biofilms: advantages and limits. Current medicinal chemistry. 2011 Jan 1;18(2):256-79. DOI: 10.2174/092986711794088399

41. Overhage J, Campisano A, Bains M, Torfs EC, Rehm BH, Hancock RE. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infection and immunity. 2008 Sep;76(9):4176-82. DOI:10.1128/IAI.00318-08

42. Bowdish DM, Davidson DJ, Hancock R. A re-evaluation of the role of host defence peptides in mammalian immunity. Current Protein and Peptide Science. 2005 Feb 1;6(1):35-51. DOI:10.2174/1389203053027494.

43. Nakatsuji T, Gallo RL. Antimicrobial peptides: old molecules with new ideas. Journal of investigative dermatology. 2012 Mar 1;132(3):887-95. DOI:10.1038/jid.2011.387

44. Tosteson MT, Holmes SJ, Razin M, Tosteson DC. Melittin lysis of red cells. The Journal of membrane biology. 1985 Feb;87:35-44. DOI: 10.1007/BF01870697.

45. Shire SJ, Shahrokh Z, Liu JU. Challenges in the development of high protein concentration formulations. Journal of pharmaceutical sciences. 2004 Jun 1;93(6):1390-402.DOI:10.1002/jps.20079.

46. Bradshaw JP. Cationic antimicrobial peptides: issues for potential clinical use. BioDrugs. 2003 Jul;17:233-40.

47. Yazici H, O'Neill MB, Kacar T, Wilson BR, Oren EE, Sarikaya M, Tamerler C. Engineered chimeric peptides as antimicrobial surface coating agents toward infection-free implants. ACS applied materials & interfaces. 2016 Mar 2;8(8):5070-81. DOI:10.1021/acsami.5b03697

48. Pletzer D, Mansour SC, Hancock RE. Synergy between conventional antibiotics and anti-biofilm peptides in a murine, sub-cutaneous abscess model caused by recalcitrant ESKAPE pathogens. PLoS pathogens. 2018 Jun 21;14(6):e1007084. DOI:10.1371/journal.ppat.1007084

49. Otvos Jr L, Ostorhazi E, Szabo D, Zumbrun SD, Miller LL, Halasohoris SA, Desai PD, Int Veldt SM, Kraus CN. Synergy between proline-rich antimicrobial peptides and small molecule antibiotics against selected gram-negative pathogens in vitro and in vivo. Frontiers in chemistry. 2018 Aug 14;6:309. DOI:10.3389/fchem.2018.00309

50. Li W, Separovic F, O'Brien-Simpson NM, Wade JD. Chemically modified and conjugated antimicrobial peptides against superbugs. Chemical Society Reviews. 2021;50(8):4932-73. DOI:10.1039/d0cs01026j

51. Pereira MP, Shi J, Kelley SO. Peptide targeting of an antibiotic prodrug toward phagosome-entrapped mycobacteria. ACS infectious diseases. 2015 Dec 11;1(12):586-92. DOI:10.1021/acsinfecdis.5b00099

52. Forde E, Devocelle M. Pro-moieties of antimicrobial peptide prodrugs. Molecules. 2015 Jan 13;20(1):1210-27. DOI:10.3390/molecules20011210

53. Brezden A, Mohamed MF, Nepal M, Harwood JS, Kuriakose J, Seleem MN, Chmielewski J. Dual targeting of intracellular pathogenic bacteria with a cleavable conjugate of kanamycin and an antibacterial cell-penetrating peptide. Journal of the American Chemical Society. 2016 Aug 31;138(34):10945-9. DOI:10.1021/jacs.6b04831

54. Low ML, Maigre L, Dorlet P, Guillot R, Pages JM, Crouse KA, Policar C, Delsuc N. Conjugation of a new series of dithiocarbazate Schiff base copper (II) complexes with vectors selected to enhance antibacterial activity. Bioconjugate Chemistry. 2014 Dec 17;25(12):2269-84. DOI:10.1021/bc5004907

55. Kumar P, Shenoi RA, Lai BF, Nguyen M, Kizhakkedathu JN, Straus SK. Conjugation of aurein 2.2 to HPG yields an antimicrobial with better properties. Biomacromolecules. 2015 Mar 9;16(3):913-23. DOI:10.1021/bm5018244

56. Zhang W, Yang X, Song J, Zheng X, Chen J, Ma P, Zhang B, Wang R. Conjugation with acridines turns nuclear localization sequence into highly active antimicrobial peptide. Engineering. 2015 Dec 1;1(4):500-5. DOI:10.15302/J-ENG-2015106

57. Ni W, Shao X, Di X, Cui J, Wang R, Liu Y. In vitro synergy of polymyxins with other antibiotics for Acinetobacter baumannii: a systematic review and meta-analysis. International journal of antimicrobial agents. 2015 Jan 1;45(1):8-18. DOI: 10.1016/j.ijantimicag.2014.10.002

58. Breidenstein EB, Courvalin P, Meziane-Cherif D. Antimicrobial activity of plectasin NZ2114 in combination with cell wall targeting antibiotics against vana-type Enterococcus faecalis. Microbial Drug Resistance. 2015 Aug 1;21(4):373-9. DOI:10.1089/mdr.2014.0221

59. Citterio L, Franzyk H, Palarasah Y, Andersen TE, Mateiu RV, Gram L. Improved in vitro evaluation of novel antimicrobials: potential synergy between human plasma and antibacterial peptidomimetics, AMPs and antibiotics against human pathogenic bacteria. Research in Microbiology. 2016 Feb 1;167(2):72-82. DOI:10.1016/j.resmic.2015.10.002

60. Tabbene O, Azaiez S, Di Grazia A, Karkouch I, Ben Slimene I, Elkahoui S, Alfeddy MN, Casciaro B, Luca V, Limam F, Mangoni ML. Bacillomycin D and its combination with amphotericin B: promising antifungal compounds with powerful antibiofilm activity and wound-healing potency. Journal of Applied Microbiology. 2016 Feb 1;120(2):289-300. DOI:10.1111/jam.13030

61. Walkenhorst WF. Using adjuvants and environmental factors to modulate the activity of antimicrobial peptides. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2016 May 1;1858(5):926-35. DOI:10.1016/j.bbamem.2015.12.034

62. Mishra B, Golla RM, Lau K, Lushnikova T, Wang G. Anti-staphylococcal biofilm effects of human cathelicidin peptides. ACS medicinal chemistry letters. 2016 Jan 14;7(1):117-21. DOI:10.1021/acsmedchemlett.5b00433

63. Čeřovský V, Bém R. Lucifensins, the insect defensins of biomedical importance: the story behind maggot therapy. Pharmaceuticals. 2014 Feb 27;7(3):251-64. DOI:10.3390/ph7030251

64. Wang G. Human antimicrobial peptides and proteins. Pharmaceuticals. 2014 May 13;7(5):545-94. DOI:10.3390/ph7050545

65. Seo MD, Won HS, Kim JH, Mishig-Ochir T, Lee BJ. Antimicrobial peptides for therapeutic applications: a review. Molecules. 2012 Oct 18;17(10):12276-86. DOI:10.3390/molecules171012276

66. Fritsche TR, Rhomberg PR, Sader HS, Jones RN. Antimicrobial activity of omiganan pentahydrochloride against contemporary fungal pathogens responsible for catheter-associated infections. Antimicrobial agents and chemotherapy. 2008 Mar;52(3):1187-9. DOI:10.1128/AAC.01475-07

67. Allen NE, Nicas TI. Mechanism of action of oritavancin and related glycopeptide antibiotics. FEMS microbiology reviews. 2003 Jan 1;26(5):511-32.DOI: https://doi.org/10.1111/j.1574-6976.2003.tb00628.x

68. Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. Journal of antimicrobial chemotherapy. 2007 Dec 1;60(6):1206-15. DOI:10.1093/jac/dkm357.