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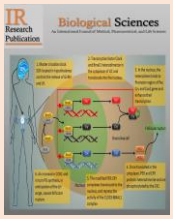

Exploring the Therapeutic Promise of Cationic Antibacterial Peptides

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ABSTRACT

The increasing global risk of antibiotic-resistant bacterial infections establishes the urgent need for novel and effective antimicrobial therapies. Cationic antimicrobial peptides (CAMPs) are a crucial component of the immediate, non-specific defence against infections in numerous species. Their potential as a new class of antimicrobials is encouraging, and ongoing research seeks to optimize their pharmacokinetics and pharmacodynamics, improve their stability, and address toxicity issues. This study examines the potential of CAMPs as novel antibacterial therapeutics, highlighting their unique structure and mechanism of action, which allows them to target bacterial membranes and disrupt their integrity, making them effective against a wide range of bacteria, including multidrug-resistant strains. Developing reliable and standardized evaluation assays, addressing regulatory and economic barriers, and advancing our comprehension of the interactions between CAMPs and the host immune system are future directions of research. CAMPs have the potential to become indispensable tools in the battle against bacterial infections if further research and development are conducted.

Introduction

Over the last 50 years, antibiotics have played a crucial role in treating bacterial diseases across the globe. However, in the past four decades, only three new antibiotic classes have been developed, leading to a shortage of antibiotics to treat Gram-negative infections (1). This scarcity, coupled with the rise of multidrug-resistant bacteria, has made it imperative to explore new antimicrobial strategies (2). Antimicrobial peptides, also known as cationic host defence peptides, have been suggested as a likely footing for a novel class of antimicrobials with clinical efficacy (3). They possess potent antimicrobial activity against a wide range of bacteria, including multidrug-resistant strains, making them a prospective source for the development of novel antibacterial therapeutics (4). This study examines the benefits and drawbacks of cationic antimicrobial peptides compared to conventional antibiotics and recent clinical developments. However, they play a crucial role in innate immunity, their net positive charge and approximately fifty percent hydrophobic residues enable them to adopt an amphiphilic structure when interacting with bacterial membranes (6, 7). Although it has been challenging to develop peptides for clinical use, advances in our knowledge of how the structure of peptides affects their mechanism of action have helped to surmount these obstacles (8).

Antibiotic resistance, which has rendered many conventional antibiotics ineffective against bacterial

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infections, has increased the need for novel antibacterial treatments (9). Therefore, developing new and effective antibacterial agents is essential (10). This article investigates the potential of cationic antimicrobial peptides as novel antibacterial therapies. To shed light on the use of CAMPs as a prospective solution to the problem of antibiotic resistance, this review article will explore the characteristics of CAMPs, their antibacterial activity, challenges and limitations, recent developments, and future directions.

Features of Cationic antimicrobial peptides (CAMPs)

They are a class of small, broad-spectrum, positively charged antibacterial peptides (11). The unique structure of cationic antimicrobial peptides (CAMPs) consists of 12 to 50 amino acids abundant in cationic and hydrophobic residues (12). High levels of positively charged amino acids endow proteins with a net positive charge, allowing them to interact with the negatively charged bacterial cell surface (13). They have a high degree of structural flexibility, allowing them to adapt to a variety of environments and shapes. This structural flexibility contributes to their capacity to penetrate bacterial membranes and compromise their integrity, causing bacterial cell demise (14). Due to their distinctive structure and properties, they are enticing candidates for the development of new antibacterial treatments (15). CAMPs are effective against gram-positive and gram-negative bacteria, as well as certain viruses and fungi. In addition, they are effective against antibiotic-resistant bacteria biofilms (16). Antimicrobial activity has been demonstrated for CAMPs against multidrug-resistant bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and carbapenem-resistant Enterobacteriaceae (17). Due to their membrane-disrupting mechanism of action, CAMPs are less susceptible to resistance development (18).

Model for the Mechanism of Action of Cationic Antimicrobial Peptides

The potential of cationic antibacterial peptides (CAPs) as therapeutics lies in their unique and multi-step mechanism of action against bacterial cells.

a) Unstructured Peptide in Solution

Many CAPs are initially unstructured in solution, allowing them to be soluble and diffuse freely in the extracellular environment. For example, Magainin from the African clawed frog (Xenopus laevis) and LL-37 from humans are unstructured in their soluble forms. This unstructured state is crucial for their initial movement and interaction with target bacteria.

b) Interaction of the Peptide with the Membrane

CAPs are attracted to bacterial membranes due to electrostatic interactions between their positively charged residues and the negatively charged components of the bacterial membrane. Cecropin from the moth (*Hyalophora cecropia*) interacts with the bacterial membrane through these electrostatic attractions. Upon approaching the membrane, CAPs like Defensins from humans adopt a more structured form, often an alpha-helix or beta-sheet, to interact effectively with the lipid bilayer. This step is critical as it initiates the direct interaction with the bacterial cell surface.

c) Integration of Peptide and Thinning of Water Leaflet

Upon binding to the membrane, CAPs insert themselves into the lipid bilayer. Magainin peptides from the African clawed frog (Xenopus laevis) exemplify this process. This integration leads to a thinning of the water layer adjacent to the membrane, increasing the peptide's interaction with the membrane lipids. The peptides typically align themselves in such a way that their hydrophobic sides face the lipid tails while the hydrophilic sides face the aqueous environment, facilitating deeper insertion into the membrane. This step is vital for destabilizing the membrane structure.

d) Aggregation/Pore Formation

As more peptides integrate into the membrane, they begin to aggregate, leading to pore formation. For example, Melittin from bee venom (Apis mellifera) aggregates to form pores. There are several models for pore formation:

- Barrel-Stave Model: Seen with Gramicidin, a peptide from Bacillus brevis, where peptides arrange themselves perpendicularly to the membrane, forming a barrel-like structure with a central pore.
- Toroidal Pore Model: Observed with LL-37 from humans, where peptides insert into the membrane and bend the lipid monolayers continuously through the pore, creating a toroid structure.
- Carpet Model: Dermaseptin from the frog (*Phyllomedusa sauvagei*) covers the membrane surface like a carpet and disrupts the membrane in a detergent-like manner, leading to micellization.

- This step is essential for creating physical disruptions in the bacterial membrane, leading to leakage of cellular contents.

e) Membrane Depolarization

The formation of pores or extensive membrane disruption leads to membrane depolarization. This disrupts the membrane potential, which is crucial for various cellular processes, including ATP production and maintenance of ionic gradients. For instance, Nisin from Lactococcus lactis causes membrane depolarization in Gram-positive bacteria. Loss of membrane potential can be immediately lethal to the cell as it collapses the electrochemical gradients required for survival. This step is crucial as it directly impacts the cell's ability to maintain homeostasis and survive.

f) Micellization, Internalization of Peptide/Diffusion to Intracellular Targets (Cell Death)

In the final stages, the membrane can be further destabilized to the point where it forms micelles, effectively disintegrating the membrane structure. LL-37 from humans can translocate across the disrupted membrane and diffuse into the cytoplasm, where it may target intracellular components like DNA, RNA, or proteins, further contributing to cell death. The combined effects of membrane disruption, loss of membrane potential, and intracellular damage lead to the rapid death of the bacterial cell.

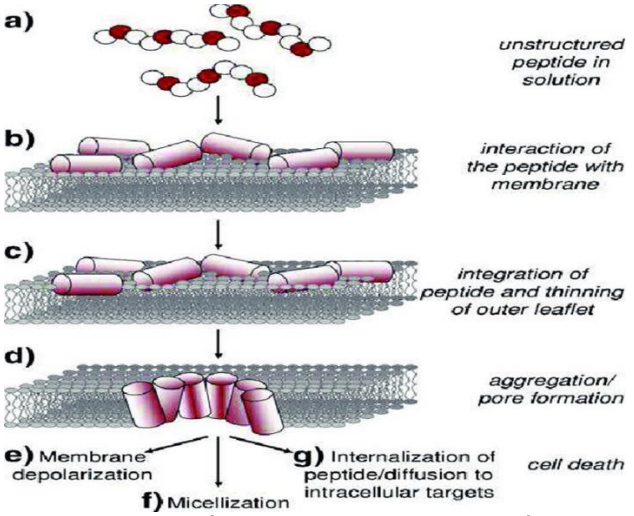

Figure 1. Model for the action mechanism of Cationic Antimicrobial Peptides. Source: (19)

Advantages of Peptides Over Conventional Antibiotics

Therapeutic peptides may be used alone to combat bacteria, in conjunction with other antibiotics to boost their efficacy, modify the immune system neutralize endotoxins, or both (16). Their adaptability is crucial to their widespread application. In particular, the most convincing substances have exceedingly broad spectrums of activity against the vast majority of gram-positive and gram-negative bacteria, fungi, and even some viruses (17). One of the greatest advantages of these antimicrobial peptides is their ability to eradicate multidrug-resistant bacteria at concentrations comparable to those required to kill susceptible infections (18). Peptides can target multiple categories of bacterial cells and eliminate pathogens significantly more quickly than conventional antibiotics (20).

Translocating across the membrane, peptides can inhibit macromolecular synthesis, activate specific enzymes or cell division, and stimulate autolysis in the cytoplasm (21). At concentrations well below the inhibitory threshold, some peptides can induce membrane permeability (22). The ratio between the minimal inhibitory concentration and the minimal bactericidal concentration is less than 2, indicating that bactericidal mortality occurs frequently (6). Peptides are unaffected by the mechanisms of resistance that threaten the efficacy of presently used antibiotics, and they exhibit excellent antibacterial activity against bacteria such as methicillin-resistant Staphylococcus aureus and multidrug-resistant *Pseudomonas aeruginosa* (23). To circumvent some of the defences developed by antibiotic-resistant microorganisms, the degradation can be utilized in conjunction with conventional antibiotics and other peptides (24).

Therapeutic peptides could be used to eliminate endotoxins, modulate the immune system, boost the effectiveness of other antibiotics, and eradicate bacteria. They have achieved extensive popularity due to their adaptability (25). The most potent agents are effective against a wide range of microorganisms, such as viruses, and the overwhelming majority of gram-negative and gram-positive bacteria, fungi, and other microorganisms (26). These antimicrobial peptides are equally effective against multidrug-resistant bacteria and susceptible pathogens at comparable concentrations. Peptides can target a broader range of bacterial cell types than conventional antibiotics and can eliminate bacteria more rapidly (27).

The ability to induce resistance is the most important aspect of any novel class of antimicrobial treatment (28). It is believed that bacteria will not develop complete resistance to cationic peptides because these peptides interact with the cytoplasmic membrane, which must alter, and because peptides may have multiple targets, making the loss of any one target less significant (6). Existing resistance mechanisms only increase resistance by a factor of

two to four. As evidence of how distinct these peptides are, it appears that bacteria cannot develop peptide cross-resistance to all peptides (29). Under the same conditions, gentamicin resistance can increase 190-fold, but it takes thirty sub-minimum inhibitory concentration peptide passages for P. aeruginosa resistance to increase two- to fourfold. However, resistance to direct selection is uncommon (30). The utilization of therapeutic peptides may induce peptide resistance in the innate immune system, making humans more susceptible to peptide-resistant bacterial infections (31). Despite the presence of peptides in over-the-counter medications (polymyxin B, gramicidin S) and consumables (nisin), neither the susceptibility of organisms to peptides nor our immune system's ability to combat bacterial infections has changed (32). In actuality, peptide-resistant bacteria such as Burkholderia, Proteus, and Serratia spp. are quite rare (33).

Endotoxemia/sepsis is a frequent and dangerous complication of bacteremia in patients receiving systemic therapy for severe bacterial infections that must be treated with antibacterial drugs administered systemically to prevent and control the spread of infection (34). In preventing sepsis and endotoxemia, peptides are superior to conventional antibiotics (35). Moreover, it has been demonstrated that specific peptides play multiple functions in the mammalian innate immune system (36). One of the most essential functions is the ability to simultaneously activate the innate immune response and suppress the potentially harmful inflammatory response (37). Animals are protected from bacterial illness by the synthetic peptide IMX00C1, which does not eradicate bacteria in test tubes (38).

Feasibility of CAMPs for Topical and Systemic Use

It has been demonstrated that CAMPs can be administered both topically and systemically. It has been considered a treatment for skin and wound diseases when found in moisturizers and ointments (26). Systemic administration of CAMPs, including intravenous or intramuscular injection, has also been studied for systemic infections, such as sepsis (16). However, there are obstacles and restrictions associated with the use of CAMPs as antibacterial medications. It can be toxic to mammalian cells at high concentrations, and their production can be difficult and expensive (17). Recent advances in CAMP research, such as the synthesis of CAMP analogs with enhanced properties, offer hope for overcoming these obstacles and expanding the use of CAMPs as novel antibacterial therapeutics (18).

Barriers to the implementation of CAMPs as antibacterial treatments

Before peptides can be used in pharmaceuticals, numerous obstacles must be surmounted. Despite the high cost of production, efforts are being made to develop peptide production platforms on a commercial scale (39). One strategy for reducing peptide size is to produce smaller, less expensive peptides to supplant larger, more expensive natural peptides. However, many peptides lose their potency under physiological sodium and serum conditions, making it difficult to achieve consistent efficacy (40). Synthetic alpha-helical peptides with modified hydrophobicity, amphipathic, charge, and alpha-helicity can be designed to circumvent this salt-dependent inactivation. Others are more precisely classified as "host defence peptides" because of their immunomodulatory properties (41).

As peptides interact with the membrane, toxicity concerns may arise; however, selectivity can be attained via lipid charge, membrane potential, and cholesterol. Potential toxicological concerns must be identified and addressed in future research (42). In addition, the pharmacodynamic and pharmacokinetic properties of systemic peptides, such as peptide aggregation, in vivo half-life, susceptibility to mammalian proteases, and optimal administration frequency, have not been thoroughly investigated (43). Inconsistencies in their efficacy and safety require the standardization of CAMP production and characterization. As the mechanism of action of CAMPs is inadequately understood, it is difficult to optimize their pharmacokinetics and pharmacodynamics. Inadequate animal models for determining the efficacy and safety of CAMPs further hinder their clinical application (44). To address these issues, it will be necessary to enhance the production and characterization of CAMPs, increase our comprehension of their mechanism of action, optimize their pharmacokinetics and pharmacodynamics, and develop animal models for assessing their efficacy and safety (45).

Adverse Effects of CAMPs (Toxicity, Stability, and Production)

Toxic effects on mammalian cells at high concentrations, rapid protease degradation, and the difficulty of mass production hinder the development of CAMPs as antibacterial therapeutics (46). However, several strategies to circumvent these restrictions are currently under investigation (47). For example, the design of synthetic CAMP analogs with enhanced stability, the development of delivery systems that protect CAMPs from degradation, and the implementation of advanced production technologies to lower costs

and increase yields (48). However, further research into the mechanism of action and animal models of CAMPs can shed light on their efficacy and safety, thereby accelerating the development of their novel antibacterial therapeutics (49).

Significant Advancements

Several research pathways have been identified to address the challenges and limitations of CAMPs as antibacterial treatments. Designing synthetic CAMP analogs with enhanced stability, activity, and selectivity is one strategy (50). Another strategy is the development of delivery systems that protect CAMPs from degradation and enhance their bioavailability and efficacy. Researchers also investigate novel CAMP sources, such as natural products or microbial communities, and optimize the production and purification of these substances (51). For the identification of novel antibacterial targets and pathways, it is also necessary to fathom the molecular mechanism of action of CAMPs (49).

They have also demonstrated efficacy when combined with conventional antibiotics and other antibacterial agents. By targeting distinct aspects of bacterial pathogenesis, combination therapies can improve efficacy, reduce toxicity, and prevent the development of resistance to both CAMPs and conventional antibiotics (52). These research avenues are essential for advancing the use of CAMPs as novel antibacterial therapeutics and combating the growing issue of antibiotic resistance. The future potential for CAMPs as novel antibacterial therapeutics is both promising and challenging (8). Several obstacles must be surmounted to maximize the therapeutic potential of CAMPs as novel antibacterial agents. Before systemic administration, the pharmacokinetics and pharmacodynamics of CAMPs must be optimized, including the determination of the optimal dose, administration route, and administration frequency (36). To assess the efficacy and safety of CAMPs in preclinical and clinical research, dependable and standardized assays must be developed. Thirdly, regulatory and economic barriers, such as intellectual property, production, and distribution, must be eliminated to facilitate the development and commercialization of CAMPs (23). Understanding the interactions between CAMPs and the host immune system and the potential for CAMPs to modulate the host's response to infection requires additional research. Despite these obstacles, CAMPs offer a promising solution to the expanding problem of antibiotic resistance (53). This field necessitates ongoing research and development to realize its maximum potential and pave the way for clinical application (36).

Advancement of Antibacterial peptides in Clinical Settings and their Industrialization

Identifying and modifying peptides derived from natural sources is essential for the development of antimicrobial peptide therapeutics. Numerous peptides developed by the pharmaceutical industry are essentially identical to their natural counterparts (54). Contrary to expectations, first-generation peptides like Pexiganan and IB367 were incapable of combating infections or substantially enhancing the efficacy of existing treatments. Xoma's injectable rBPI21 showed only a hint of efficacy in Phase III data (55). Recent efforts have centred on lowering expenses by employing smaller molecules that retain the physiologically active core of the native peptide (56).

MX-226 is the only topical antibiotic that has demonstrated efficacy in Phase III clinical trials. (also known as CPI-226). Utilized to prevent catheter infections, this peptide is derived from bovine indolicidin (57). Catheter colonization was reduced by 21%, and catheter site infections were reduced by 49%, according to Phase III studies involving MX-226. Cadence is conducting a Phase IIIb confirmatory trial to assess the effectiveness of MX-226 in preventing CAIs (58). Other clinical trials include Phase I trials to prevent infections in patients undergoing an allogeneic stem cell transplant and Phase II trials to treat mild-to-moderate acne (36).

Antimicrobial peptides are utilized to treat neutropenia and cystic fibrosis patients. Two formulations are administered topically, while the other two are ingested (36). For the topical treatment of infections caused by P. aeruginosa and Acinetobacter baumannii, antimicrobials such as polymyxins and gramicidin S are advantageous because they are clinically safe, effective, and rarely result in the development of resistance (59). However, systemic administration of clinically significant concentrations would be too dangerous. Lung infections caused by cystic fibrosis are treated intravenously with oligomycin, a prodrug of colistin (60). Infections of the epidermis and its supporting structures can be caused by numerous bacteria; however, daptomycin is effective against Streptococcus pyogenes Streptococcus dysgalactiae subspecies equisimilis, Enterococcus faecalis, Streptococcus agalactiae and Staphylococcus aureus strains (61, 62). Ca2+ is required for daptomycin to have the same membrane effect as antimicrobial peptides. The curability of experimental pneumococcal meningitis has been demonstrated (36, 63). Attempts to reduce the toxicity of polymyxins and gramicidin have been made, but no second-generation pharmaceuticals have been developed as of yet. Bacitracin, polymyxin b, and gramicidin s are frequently

combined in the majority of wound treatments, eye drops, and ear drops (36,64).

Conclusion

Cationic antimicrobial peptides (CAMPs) possess several characteristics that make them promising antibacterial therapy candidates. Their structure and mechanism of action enable them to target bacterial membranes and disrupt their integrity, rendering them effective against a wide range of bacteria, including those resistant to multiple antibiotics. Despite the need to surmount certain limitations and obstacles, CAMPs have demonstrated promise for topical and systemic administration. Recent advances in CAMP research and development have centred on optimizing their pharmacokinetics and pharmacodynamics, enhancing their stability, and addressing toxicity concerns. Combining CAMPs with conventional antibiotics and other antibacterial agents has proven effective. By combining CAMPs with other antibacterial agents, the efficacy of these interventions could be increased while the risk of resistance development is diminished. Throughout the study, the therapeutic potential of CAMPs as novel antibacterial agents is emphasized. CAMPs are a class of antimicrobial agents with the potential to combat antibiotic resistance. It has the potential to become an essential weapon against bacterial infections if further research and development are conducted. Future research directions in the field of CAMPs as antibacterial therapies include optimizing their pharmacokinetics and pharmacodynamics, developing reliable and standardized evaluation assays, addressing regulatory and economic barriers, and expanding our knowledge of CAMP-host immune interactions. Further research is required to realize the full potential of CAMPs and pave the way for their clinical application.

Contribution of authors

Not Applicable

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Conflict of Interest

The author declares no conflict of interest.

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References

1. Griffith, A., Mateen, A., Markowitz, K., Singer, S. R., Cugini, C., Shimizu, E & Kumar, V. (2022). Alternative antibiotics in dentistry: Antimicrobial peptides. *Pharmaceutics*, *14*(8), 1679.
2. Lin, B., Li, R., Handley, T. N., Wade, J. D., Li, W., & O'Brien-Simpson, N. M. (2021). Cationic antimicrobial peptides are leading the way to combat uropathogenic infections. *ACS Infectious Diseases*, *7*(11), 2959-2970.
3. Aisenbrey, C., Marquette, A., & Bechinger, B. (2019). The mechanisms of action of cationic antimicrobial peptides are refined by novel concepts from biophysical investigations. *Antimicrobial Peptides: Basics for Clinical Application*, 33-64.
4. Browne, K., Chakraborty, S., Chen, R., Willcox, M. D., Black, D. S., Walsh, W. R., & Kumar, N. (2020). A new era of antibiotics: the clinical potential of antimicrobial peptides. *International journal of molecular sciences*, *21*(19), 7047.
5. Mookherjee, N., Anderson, M. A., Haagsman, H. P., & Davidson, D. J. (2020). Antimicrobial host defense peptides: functions and clinical potential. *Nature reviews Drug discovery*, *19*(5), 311-332.
6. Zhu, Y., Hao, W., Wang, X., Ouyang, J., Deng, X., Yu, H., & Wang, Y. (2022). Antimicrobial peptides, conventional antibiotics, and their synergistic utility for the treatment of drug-resistant infections. *Medicinal Research Reviews*, *42*(4), 1377-1422.
7. van der Does, A. M., Hiemstra, P. S., & Mookherjee, N. (2019). Antimicrobial host defense peptides: immunomodulatory functions and translational prospects. *Antimicrobial Peptides: Basics for Clinical Application*, 149-171.
8. Magana, M., Pushpanathan, M., Santos, A. L., Leanse, L., Fernandez, M., Ioannidis, A & Tegos, G. P. (2020). The value of antimicrobial peptides in the age of resistance. *The Lancet Infectious Diseases*, *20*(9), e216-e230.
9. Mahlapuu, M., Björn, C., & Ekblom, J. (2020). Antimicrobial peptides as therapeutic agents: Opportunities and

challenges. *Critical reviews in biotechnology*, *40*(7), 978-992.
10. Deslouches, B., Montelaro, R. C., Urish, K. L., & Di, Y. P. (2020). Engineered cationic antimicrobial peptides (eCAPs) to combat multidrug-resistant bacteria. *Pharmaceutics*, *12*(6), 501.
11. Valenti, G. E., Alfei, S., Caviglia, D., Domenicotti, C., & Marengo, B. (2022). Antimicrobial peptides and cationic nanoparticles: a broad-spectrum weapon to fight multi-drug resistance not only in bacteria. *International Journal of Molecular Sciences*, *23*(11), 6108.
12. Leon Madrazo, A., & Segura Campos, M. R. (2020). Review of antimicrobial peptides as promoters of food safety: Limitations and possibilities within the food industry. *Journal of Food Safety*, *40*(6), e12854.
13. Tornesello, A. L., Borrelli, A., Buonaguro, L., Buonaguro, F. M., & Tornesello, M. L. (2020). Antimicrobial peptides as anticancer agents: functional properties and biological activities. *Molecules*, *25*(12), 2850.
14. Seyfi, R., Kahaki, F. A., Ebrahimi, T., Montazersaheb, S., Eyvazi, S., Babaeipour, V., & Tarhriz, V. (2020). Antimicrobial peptides (AMPs): roles, functions, and mechanism of action. *International Journal of Peptide Research and Therapeutics*, *26*, 1451-1463.
15. Liu, Y., Shi, J., Tong, Z., Jia, Y., Yang, B., & Wang, Z. (2021). The revitalization of antimicrobial peptides in the resistance era. *Pharmacological Research*, *163*, 105276.
16. Yang, M., Zhang, C., Zhang, M. Z., & Zhang, S. (2018). Beta-defensin-derived cationic antimicrobial peptides with potent killing activity against gram-negative and gram-positive bacteria. *BMC Microbiology*, *18*(1), 1-14.
17. Grant, T. M., Rennison, D., Krause, A. L., Mros, S., Ferguson, S. A., Cook, G. M & Svenson, J. (2022). Stereochemical Effects on the Antimicrobial Properties of Tetrasubstituted 2, 5-Diketopiperazines. *ACS Medicinal Chemistry Letters*, *13*(4), 632-640.
18. Wang, X., Sun, Y., Wang, F., You, L., Cao, Y., Tang, R. & Cui, X. (2020). A novel endogenous antimicrobial peptide CAMP 211-225 derived from casein in human milk. *Food & Function*, *11*(3), 2291-2298.
19. Devine, D. A., & Hancock, R. E. (2002). Cationic peptides: distribution and mechanisms of resistance. *Current pharmaceutical design*, *8*(9), 703-714.
20. da Cunha, N. B., Cobacho, N. B., Viana, J. F., Lima, L. A., Sampaio, K. B., Dohms, S. S & Dias, S. C. (2017). The next generation of antimicrobial peptides (AMPs) as molecular therapeutic tools for the treatment of diseases with social and economic impacts. *Drug Discovery Today*, *22*(2), 234-248.
21. Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., ... & He, Q. (2019). The antimicrobial peptides and their potential clinical applications. *American journal of translational research*, *11*(7), 3919.
22. Pervin, Z., & Hassan, M. M. (2021). Synergistic therapeutic actions of antimicrobial peptides to treat multidrug-resistant bacterial infection. *Reviews and Research in Medical Microbiology*, *32*(2), 83-89.
23. Cardoso, M. H., Meneguetti, B. T., Costa, B. O., Buccini, D. F., Oshiro, K. G., Preza, S. L & Franco, O. L. (2019). Non-lytic antibacterial peptides that translocate through bacterial membranes to act on intracellular targets. *International Journal of Molecular Sciences*, *20*(19), 4877.
24. Um, J., Manguy, J., Anes, J., Jacquier, J. C., Hurley, D., Dillon, E. T., ... & Shields, D. C. (2021). Enriching antimicrobial peptides from milk hydrolysates using pectin/alginate food-gels. *Food Chemistry*, *352*, 129220.
25. Malanovic, N., & Lohner, K. (2016). Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1858*(5), 936-946.
26. Lima, B., Ricci, M., Garro, A., Juhász, T., Szigyártó, I. C., Papp, Z. I., & Enriz, R. D. (2021). New short cationic antibacterial peptides. Synthesis, biological activity, and mechanism of action. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1863*(10), 183665.
27. Ageitos, J. M., Sánchez-Pérez, A., Calo-Mata, P., & Villa, T. G. (2017). Antimicrobial peptides (AMPs): Ancient compounds that represent novel weapons in the fight against bacteria. *Biochemical pharmacology*, *133*, 117-138.
28. Lee, E. Y., Lee, M. W., & Wong, G. C. (2019, April). Modulation of toll-like receptor signaling by antimicrobial peptides. In *Seminars in cell & developmental biology* (Vol. 88, pp. 173-184). Academic Press.
29. Ramesh, S., Govender, T., Kruger, H. G., de

la Torre, B. G., & Albericio, F. (2016). Short AntiMicrobial Peptides (SAMPs) as a class of extraordinarily promising therapeutic agents. *Journal of Peptide Science*, *22*(7), 438-451.
30. Kosikowska, P., & Lesner, A. (2016). Antimicrobial peptides (AMPs) as drug candidates: a patent review (2003–2015). *Expert opinion on therapeutic patents*, *26*(6), 689-702.
31. Haney, E. F., Mansour, S. C., & Hancock, R. E. (2017). Antimicrobial peptides: an introduction. *Antimicrobial peptides: methods and protocols*, 3-22.
32. Bechinger, B., & Gorr, S. U. (2017). Antimicrobial peptides: mechanisms of action and resistance. *Journal of dental research*, *96*(3), 254-260.
33. Juárez-López, D., Morales-Ruiz, E., Herrera-Zúñiga, L. D., González-Carrera, Z., Cuevas-Reyes, E., Corzo, G., ... & Villegas, E. (2023). The Resilience of Pseudomonas aeruginosa to Antibiotics and the Designing of Antimicrobial Peptides to Overcome Microbial Resistance. *Current Medicinal Chemistry*, *30*(1), 72-103.
34. Maria-Neto, S., de Almeida, K. C., Macedo, M. L. R., & Franco, O. L. (2015). Understanding bacterial resistance to antimicrobial peptides: From the surface to deep inside. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1848*(11), 3078-3088.
35. Strempel, N., Strehmel, J., & Overhage, J. (2015). Potential application of antimicrobial peptides in the treatment of bacterial biofilm infections. *Current pharmaceutical design*, *21*(1), 67-84.
36. Marr, A. K., Gooderham, W. J., & Hancock, R. E. (2006). Antibacterial peptides for therapeutic use: obstacles and realistic outlook. *Current opinion in pharmacology*, *6*(5), 468-472.
37. Solov'eva, T., Davydova, V., Krasikova, I., & Yermak, I. (2013). Marine compounds with therapeutic potential in gram-negative sepsis. *Marine drugs*, *11*(6), 2216-2229.
38. Brandenburg, K., Heinbockel, L., Correa, W., & Lohner, K. (2016). Peptides with dual mode of action: Killing bacteria and preventing endotoxin-induced sepsis. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1858*(5), 971-979.
39. Kościuczuk, E. M., Lisowski, P., Jarczak, J., Strzałkowska, N., Jóźwik, A., Horbańczuk, J., ... & Bagnicka, E. (2012). Cathelicidins: the family of antimicrobial peptides. A review. *Molecular biology reports*, *39*, 10957-10970.
40. Scott, M. G., & Hancock, R. E. (2000). Cationic antimicrobial peptides and their multifunctional role in the immune system. *Critical Reviews™ in Immunology*, *20*(5). 407-31.
41. Ahmed, S., Ahmed, M. Z., Rafique, S., Almasoudi, S. E., Shah, M., Jalil, N. A. C., & Ojha, S. C. (2023). Recent Approaches for Downplaying Antibiotic Resistance: Molecular Mechanisms. *BioMed Research International*, *2023* (1), 5250040.
42. Weghuber, J., Aichinger, M. C., Brameshuber, M., Wieser, S., Ruprecht, V., Plochberger, B., & Schütz, G. J. (2011). Cationic amphipathic peptides accumulate sialylated proteins and lipids in the plasma membrane of eukaryotic host cells. *Biochimica et Biophysica Acta (BBA)-Biomembranes*,*1808*(10), 2581-2590.
43. Teixeira, V., Feio, M. J., & Bastos, M. (2012). Role of lipids in the interaction of antimicrobial peptides with membranes. *Progress in lipid research*, *51*(2), 149-177.
44. Alfei, S., & Schito, A. M. (2020). From nanobiotechnology, positively charged biomimetic dendrimers as novel antibacterial agents: A review. *Nanomaterials*, *10*(10), 2022.
45. Barreto-Santamaría, A., Patarroyo, M. E., & Curtidor, H. (2019). Designing and optimizing new antimicrobial peptides: All targets are not the same. *Critical Reviews in Clinical Laboratory Sciences*, *56*(6), 351-373.
46. Sharma, K., Aaghaz, S., Shenmar, K., & Jain, R. (2018). Short antimicrobial peptides. *Recent patents on anti-infective drug discovery*, *13*(1), 12-52.
47. Hadley, B. E., & EW Hancock, R. (2010). Strategies for the discovery and advancement of novel cationic antimicrobial peptides. *Current topics in medicinal chemistry*, *10*(18), 1872-1881.
48. Kang, S. J., Park, S. J., Mishig-Ochir, T., & Lee, B. J. (2014). Antimicrobial peptides: therapeutic potentials. *Expert review of anti-infective therapy*, *12*(12), 1477-1486.
49. Onaizi, S. A., & Leong, S. S. (2011). Tethering antimicrobial peptides: current status and potential challenges. *Biotechnology advances*, *29*(1), 67-74.
50. Kundu, R. (2020). Cationic amphiphilic peptides: Synthetic antimicrobial agents inspired by nature. *ChemMedChem*, *15*(20), 1887-1896.
51. Guilhelmelli, F., Vilela, N., Albuquerque, P., Derengowski, L. D. S., Silva-Pereira, I., &

Kyaw, C. M. (2013). Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and bacterial resistance. *Frontiers in microbiology*, *4*, 353.
52. Gera, S., Kankuri, E., & Kogermann, K. (2022). Antimicrobial peptides–unleashing their therapeutic potential using nanotechnology. *Pharmacology & Therapeutics*, *232*, 107990.
53. Lin, S., Wade, J. D., & Liu, S. (2020). De novo design of flavonoid-based mimetics of cationic antimicrobial peptides: Discovery, development, and applications. *Accounts of Chemical Research*, *54*(1), 104-119.
54. Ciumac, D., Gong, H., Hu, X., & Lu, J. R. (2019). Membrane targeting cationic antimicrobial peptides. *Journal of colloid and interface science*, *537*, 163-185.
55. Schulze, M., Dathe, M., Waberski, D., & Müller, K. (2016). Liquid storage of boar semen: Current and future perspectives on the use of cationic antimicrobial peptides to replace antibiotics in semen extenders. *Theriogenology*, *85*(1), 39-46.
56. Jafari, A., Babajani, A., Sarrami Forooshani, R., Yazdani, M., & Rezaei-Tavirani, M. (2022). Clinical applications and anticancer effects of antimicrobial peptides: From bench to bedside. *Frontiers in Oncology*, *12*, 350.
57. Wang, J., Dou, X., Song, J., Lyu, Y., Zhu, X., Xu, L & Shan, A. (2019). Antimicrobial peptides: Promising alternatives in the post-feeding antibiotic era. *Medicinal research reviews*, *39*(3), 831-859.
58. Koo, H. B., & Seo, J. (2019). Antimicrobial peptides under clinical investigation. *Peptide Science*, *111*(5), e24122.
59. Jiang, Y., Chen, Y., Song, Z., Tan, Z., & Cheng, J. (2021). Recent advances in the design of antimicrobial peptides and polypeptides toward clinical translation. *Advanced Drug Delivery Reviews*, *170*, 261-280.
60. Giuliani, A., & Rinaldi, A. C. (2011). Beyond natural antimicrobial peptides: multimeric peptides and other peptidomimetic approaches. *Cellular and Molecular Life Sciences*, *68*, 2255-2266.
61. Gan, B. H., Gaynord, J., Rowe, S. M., Deingruber, T., & Spring, D. R. (2021). The multifaceted nature of antimicrobial peptides: Current synthetic chemistry approaches and future directions. *Chemical Society Reviews*, *50*(13), 7820-7880.
62. Kalelkar, P. P., Riddick, M., & García, A. J. (2022). Biomaterial-based antimicrobial therapies for the treatment of bacterial infections. *Nature Reviews Materials*, *7*(1), 39-54.
63. Wang, L., Qu, L., Lin, S., Yang, Q., Zhang, X., Jin, L & Sun, D. (2022). Biological Functions and Applications of Antimicrobial Peptides. *Current Protein and Peptide Science*, *23*(4), 226-247.
64. Mohammed, I., Said, D. G., & Dua, H. S. (2017). Human antimicrobial peptides in ocular surface defense. *Progress in retinal and eye research*, *61*, 1-22.