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Review Article

Recombinant Antimicrobial Peptides (rAMPs); Potential Applications in Medicine and Veterinary Medicine: A Review

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Abstarct: Antibiotic resistance has become a major public health concern worldwide. Treatment of humans and animals is becoming increasingly challenging due to antibiotic resistance. Antibiotic-resistant bacteria can be transmitted from animals to humans by several routes, including direct contact, contaminated food or water, or environmental exposure. Various factors contribute to the rising problem, such as the widespread and indiscriminate exploitation of antimicrobials in both human and animal healthcare, over-prescription, misuse of antibiotics, the role of agriculture in spreading antibiotic resistance, and poor animal husbandry practices. According to the preliminary findings, recombinant antimicrobial peptides are an interesting novel area of biotechnology and medical innovation that might be employed as a secure and effective substitute for antibiotics. In this review study, we briefly examine the factors contributing to the rise of antibiotic resistance. We then introduce and discuss recombinant antimicrobial peptides as a promising strategy to address this growing problem.

Keywords: Antimicrobial peptides, Antibiotic resistance, Recombinant, Veterinary medicine.

1. Background

Antimicrobial resistance (AMR) is an international concern that causes a major threat to animal and public well-being. Disease treatments in the fields of human and veterinary medicine are becoming increasingly challenging due to antibiotic resistance, a type of AMR. Antibiotic-resistant bacteria can be transmitted from animals to humans by several routes, including direct contact, contaminated food or water, or environmental exposure (1). Additionally, these bacteria have the ability to exchange genetic materials with other bacteria, resulting in the development of new antibiotic-resistant strains (2). This phenomenon makes it more difficult, if not impossible, to cure infections with currently available antibiotics. Antibiotic-resistant infections are thought to be responsible for 700,000 annual fatalities, which has a staggering effect on the world. By 2050, if current trends continue, this figure might increase to

10 million fatalities annually (3). Antibiotic resistance has a substantial financial impact as well. According to projections, it might cost the world economy up to 100 trillion dollars by 2050. Overuse and misuse of antibiotics in food animals in veterinary medicine is a major factor influencing the development and spread of antibiotic-resistant infections (4).

In this article, we briefly explore the factors contributing to the rise of antibiotic resistance in veterinary medicine. Then we introduce and discuss recombinant antimicrobial peptides as a promising strategy to address this growing issue.

2. Antibiotic Resistance

Antibiotic resistance has been present before even antimicrobials existed. Bacteria isolated from 2000 years ago carried resistance to ampicillin, and some other from the permafrost which was more than 30,000 years ago had vancomycin-resistant genes (5). At least 700,000 deaths per year are related to drug-resistant infections at the moment. World Health Organization (WHO) published a report saying this figure can increase to 10 million deaths annually if no action is taken (6). There are many reports of the high prevalence of antibiotic resistance in Iran, including hospital-acquired infections and the prevalence of microbial resistance in medical and veterinary treatment centers. These results are based on information from medical, veterinary, and research diagnostic laboratories, which include medical and veterinary collections (7-10).

3. Antibiotic Resistance in Medicine

Antibiotic resistance is an important issue that is growing dangerously in Iran. For example, during a study conducted by Saeli *et al*. (11), 200 *Pseudomonas aeruginosa* isolates were obtained from five hospitals. Their results showed that 48% of the isolates were resistant to aminoglycosides. In another study, 568 bacteria were isolated from patients with clinical symptoms of urinary tract infection in Sari, Avicenna Hospital. Antibiogram assessment showed that 87.5% of bacteria were resistant to at least one antibiotic (12). In a study, 113 isolates of *Enterobacter cloacae* were collected from clinical samples in Kermanshah, Iran. The frequency of plasmid-mediated quinolone resistance (PMQR) genes was 79.6% (13). Narimisa *et al*. (14) examined 18 studies about antibiotic resistance in *Salmonella typhimurium* isolates in Iran. According to their research, the highest resistance rate was reported in piperacillin and tetracycline. In another study conducted in Rasht, Iran, patients with septicemia were examined, and blood culture was done. Various organisms were isolated, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The highest resistance was observed to ampicillin, ceftriaxone, and ceftizoxime. It must be noted that these antibiotics are commonly used in Iranian hospitals (15). Besides, it has been demonstrated that antibiotic resistance in *Escherichia coli* is rising in Iran (16).

4. Antibiotic Resistance in Veterinary Medicine

Over the past 70 years, the use of antibiotics in animals raised for human consumption has become widespread. Antibiotic resistance has become a significant problem in veterinary medicine over the past few decades. Some important factors are described here.

4.1. Antibiotic Misuse and Overuse

The overuse and misuse application of antibiotics in human and animal healthcare is a major contributing factor to antibiotic resistance (17). Sometimes the use of antibiotics is also prolonged unnecessarily, increasing the number of bacteria exposed to the drugs and, in turn, contributing to the development of antibiotic resistance. In addition, veterinarians choose some antibiotics over others mainly because they are more effective, which leads to overprescription (18)

4.2. The Role of Agriculture

The growing application of antibiotics in agriculture for the prevention of diseases and the promotion of growth contributes significantly to the spread of antibiotic resistance. In some countries, such as China, India, and Brazil, antibiotics are extensively used in livestock farming without proper regulation or oversight. The widespread use of antibiotics in agriculture creates ideal conditions for bacterial adaptation and the development of antibiotic-resistant strains (19).

4.3. Animal Husbandry

Antimicrobials, such as antibiotics, antifungals, and antiprotozoals, are now widely used in industrial agriculture to cure and prevent disease, enhance animal welfare, and boost productivity. It has been clear in recent years that the widespread use of antibiotics in animals has aided in the development of antibiotic resistance and, as a result, limited the range of possible treatments (20). Poor sanitation, inadequate ventilation, overcrowding, a lack of vaccination programs, and early weaning are all variables that can cause stress in animals, thereby increasing their susceptibility to infection. As a result, the widespread use of unnecessary and inappropriate antibiotics promotes antibacterial resistance (21). Due to an increase in the demand for animal protein worldwide, the use of antibiotics in livestock has risen in recent decades. It has been stated that almost 73% of antimicrobials sold worldwide are meant for use in food-producing animals (22). In addition to treating infectious diseases, these antibiotics are also used at sub-therapeutic levels to promote growth and prevent disease (prophylactic use: giving antibiotics to animals that are highly susceptible to illness; metaphylactic use: treating a group of animals that are in close proximity but do not show signs of illness). The use of fluoroquinolones in food-producing animals has been linked to resistance in *E. coli*, *Salmonella spp*., *Campylobacter jejuni*, and *Campylobacter coli* in both humans and animals (23).

There are numerous studies about antibiotic resistance in veterinary medicine. During a study, researchers utilized the conventional procedure of disc diffusion to assess the antibiotic susceptibility profile of enterococci, isolated from chicken and companion birds. The results demonstrated that all *E. faecalis* and *E. faecium* isolates showed resistance against more than five antibiotics. Cefazolin, tiamulin, flumequine, and cephalexin resistance were identified in the vast majority of *E. faecalis* and *E. faecium* isolates (4). In another study, 100 white and feta cheese samples were obtained from various suppliers. The acquired isolates were tested for *mecA* gene presence using PCR and disc diffusion antimicrobial susceptibility assays. When evaluated using the disc diffusion method, 23 (92%) of the 25 isolates showed resistance to at least one antibiotic and perhaps more. Penicillin G was shown to have the highest rate of antibiotic resistance (92%), followed by ampicillin (73%), and cloxacillin (68%) (24). In another report, it was observed that all *Clostridium perfringens* strains isolated from fresh and non-frozen chicken neck, wing, liver, and gizzard from protein material supply centers in Mashhad were resistant to traditional antibiotics, such as erythromycin, gentamicin, and cloxacillin (25). In another study, *Pseudomonas aeruginosa* was isolated from 126 companion birds. The highest rate of resistance was shown against neomycin, kanamycin, rifampicin, and vancomycin (100% of isolates), followed by colistin (57% of isolates) (26). Also, antibiotic resistance has been observed in *Klebsiella pneumoniae*, isolated from common canary, and *Streptococcus spp*. (27, 28). Resistance to β-lactam and aminoglycoside antibiotics seems to be prevalent among different sources of bacteria, so it should be prescribed with caution. It has been found that oral administration of antimicrobial peptides, had beneficial effects on stress response and reduced the cortisol, inflammatory factors (TNF-α and IL-1β), and diarrhea rate in Ragdoll cats (29). In addition, adding antimicrobial peptides to the diet of sheep (2.5 g/sheep/day) has been able to significantly increase the daily weight gain and decrease the feed conversion ratio in comparison to the control group (30).

Viral infection of pigs is among the most important issues in the pig industry worldwide. It has been indicated that some antimicrobial peptides, including Piscidin-1, Caerin 1.1, pBD-2, pBD-3, Cecropin D, Cecropin P1, Protegrin-1, Protegrin-4, LL-37, Epinecidin-1, DNBLK1 can be used against porcine reproductive and respiratory syndrome virus (PRRSV), porcine epidemic diarrhea virus (PEDV), and pseudorabies virus (PRV) (31).

5. Strategies To Combat Antibiotic Resistance

Veterinarians can prescribe antibiotics in accordance with the standards established by the relevant veterinary associations to combat this growing issue. The prescription of such medication should only be based on diagnostic results. Additionally, whenever possible, the application of broader-spectrum antibiotics can be prevented because this promotes resistance. To reduce antibiotic resistance in all facets, the use of growth-promoting antibiotics should be minimized or banned in animal husbandry. By applying proper animal husbandry practices, stress levels in animals can be lowered to avoid infections. Finally, there needs to be more focus on the promotion of new antibiotic substitutes with distinct modes of action (32). In addition, several methods exist for controlling microbial resistance, including 1) effective diagnosis and treatment of infection; 2) rational antimicrobial use; 3) surveillance of antibiotic resistance and antibiotic use; 4) improving the antimicrobial quality and supply chain; and 5) good microbiology practices. Recombinant antimicrobial peptides (AMPs) are one such promising alternative therapy used in human or veterinary medicine. In recent years, there has been growing interest in the therapeutic potential of AMPs for the treatment of infectious diseases.

6. Antibiotics Side Effects

Gastrointestinal disturbances are amongst the most common side effects of antibiotics which can include diarrhea, nausea, vomiting, and abdominal pain (33, 34). This results from the disruption of the regular gut microbiota, which may cause the proliferation of harmful bacteria like *Clostridium difficile* (35). Allergy reactions, which can vary from minor skin rashes to potentially fatal anaphylaxis, are another antibiotic adverse effect. Allergic reactions are more common with certain types of antibiotics, such as penicillins and cephalosporins (34). Furthermore, antibiotics can adversely affect the body's organs including the liver and kidneys, especially in individuals who already have hepatic diseases, renal impairment, or are taking several medications (36).

7. Antimicrobial Peptides (AMPs)

A vast variety of small molecules known as antimicrobial peptides (AMPs) have significant antimicrobial activity against various kinds of pathogens, including bacteria, fungi, viruses, parasites, and antibiotic-resistant microorganisms (37). It has been demonstrated that AMPs, as a member of innate immunity, are key factors in the initial defense line against invasive infections. AMPs usually contain 12–50 amino acid residues, have a net positive charge and an amphipathic structure (38). The first AMP was recognized in the American silkworm chrysalis. After that, nisin was the first AMP isolated from bacteria. To date, more than 3700 AMPs have been reported from different organisms worldwide. In this regard, defensins, cathelicidins, and histatins are a few examples (39). AMPs present as prospective substitutes for conventional antibiotics. AMPs can be used in a variety of human and veterinary medical applications (40). For instance, they could be employed in combating bacterial infections, including methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant bacteria that are resistant to conventional antibiotics. In high-risk populations such as immunocompromised individuals or those with invasive medical procedures, AMPs may also be used to prevent infections.

8. Recombinant Antimicrobial Peptides: A Novel Approach for Combating AMR

Nevertheless, despite their potent antimicrobial activity, the majority of AMPs have limited clinical utility due to a number of stability, efficacy, and toxicity concerns. A possible solution to these issues is provided by recombinant antimicrobial peptides (rAMPs). rAMPs, a promising new class of medicines, have shown considerable promise in preclinical investigations and have the potential to confront the increasing phenomenon of antibiotic-resistant infections. Researchers can produce recombinant peptides that retain substantial antimicrobial activity while also being appropriate for therapeutic usage through the genetic modification of AMPs. As the technology continues to evolve and improve, rAMPs will undoubtedly play a critical role in improving healthcare outcomes both locally and globally. Antibiotics used to enhance the growth of chickens could be replaced with recombinant peptides (41).

rAMPs have broad-spectrum activity, in contrast to conventional antibiotics, which focus on particular bacterial metabolic pathways (42, 43).

It has been demonstrated that the AMPs mode of action can be generally divided into the membrane model and the intracellular model. In the membrane model, the AMP binds to the anionic cytoplasmic membrane and forms a peptide-lipid complex micelle, which results in intracellular material efflux and cell death. In other words, AMPs can act like surfactants and rupture the membrane. In the intracellular model, AMPs can have effects on nucleic acids (such as Buforin II and indolicidin), protein synthesis (such as PR-39 and Api137), activity of enzymes (like pyrrhocoricin and microcin J25), and synthesis of cell walls (HNP1) (39).

Similar to naturally occurring AMPs, rAMPs cause cell death by compromising the pathogen's cell membrane integrity. For instance, AMP-17, an antimicrobial peptide obtained from *Musca domestica*, exhibits excellent antimicrobial properties against *Candida albicans* by compromising the integrity of the cell wall and membrane (44). In addition, rAMPs also have immunomodulatory features that can improve the host immune system's defense. For instance, rAMPs can trigger the synthesis of cytokines and chemokines,

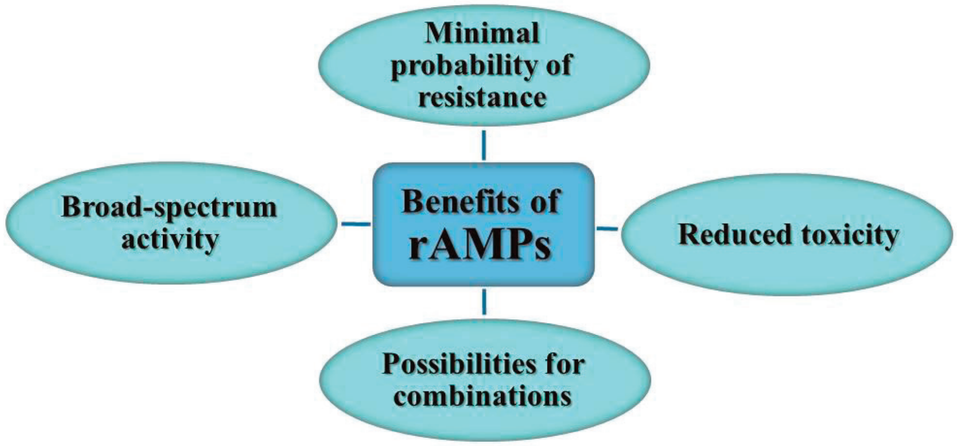

Figure 1. Potential benefits of rAMPs

which recruit immune cells to the site of infection and improve their capacity to eradicate infections (42).

A better-balanced microbial community was seen as a result of a precision-guided AMP's ability to target harmful bacteria while preserving the beneficial microbiota (45). Researchers can increase the efficacy and selectivity of antimicrobial peptides against specific pathogens, such as Gram-negative or biofilm-producing bacteria, by modifying their amino acid sequence or fusing different peptides. For instance, Choudhury (46) linked the guiding peptide (A12C) to the AMPs eurocin and plectasin, and the guided-AMPs (gAMPs) were expressed in *Escherichia coli*. The guided AMPs were found to exhibit high selective suppression of *S. aureus* as a preliminary pathogen but to be considerably less toxic against different off-target bacteria.

There are several potential benefits associated with the use of rAMPs for treating bacterial infections **(Fig. 1)**:

- *Broad-spectrum activity:* Some rAMPs, such as cLF36, have shown activity against a great number of bacteria, including those that are resistant to multiple antibiotics (47).
- *Minimal probability of resistance*: Because rAMPs act on bacterial membranes, they are less likely to elicit resistance than antibiotics that target specific cellular pathways.
- *Possibilities for combinations*: rAMPs can be combined with other drugs or therapies to enhance their efficacy, potentially reducing the risk of resistance development. For example, an in vitro study conducted by Rashidian *et al*. (48) indicated that two antimicrobial peptides, nisin, and recombinant CLF36, have synergistic effects and could be used together.
- *Reduced toxicity*: By engineering AMPs to reduce their toxicity, researchers can create molecules that are better tolerated by patients.

9. AMPs Applications

rAMPs are also incredibly versatile and can be used across a range of applications. The potential uses for rAMPs are virtually limitless. Here are some of the most exciting applications of AMPs:

9.1. Bioengineered Probiotics

Application of transgenic probiotics, containing recombinant antimicrobial peptides, for treating gastrointestinal infections.

9.2. Food Preservation

Foodborne infections continue to be a major public health concern. Due to their broad-spectrum antibacterial activity, AMPs have shown potential as natural food preservatives (49). For example, Ac-AMP2, a plant AMP initially produced by the annual flowering plant *Amaranthus caudatus*, and MiAMP1, a highly basic protein from the nut kernel of *Macadamia integrifolia*, were both confirmed, expressed, and produced using *Pichia pastoris* by Huang *et al*. (50)

Table 1. Recent experiments on the usage and effects of antimicrobial peptides in animal models.

AMP name	Origin of AMP	Amino acid sequence	Animal model	Results	Infection	Ref.
HJH-3	Bovine erythrocyte P3	VNFKLL-SHSLLV-TLRSHL	Chicken, IP injection (200 mg.mL^{-1}, 0.5 mL)., challenge with *Salmonella Pullorum*	Protection of chickens (100 % survival rate) from lethal challenge with *Salmonella* Pullorum	Pullorum disease (PD)	(74)
Microcin C7	*Escherichia coli* cells harboring a plasmid-borne mccABCDE	MRTGNAD	Chicken, basal diet supplemented with 2, 4, and 6 mg.kg^{-1} Microcin C7.	Microcin C7 significantly: increased the levels of serum cytokine IL-10, IgG, IgM, and sIgA. Microcin C7 significantly increased villus height and decreased crypt depth in small intestine. Microcin C7 increased gene expression of tight junction protein and decreased gene expression of pro-inflammatory cytokines. Microcin C7 increased the number of *Lactobacillus* and decreased the number of total bacteria in the cecum.	×	(75)
MetchnikowinII (MetII)	Drosophila	VDKPD-YRPRP-WPRPN	Chicken, IM injection, Challenge with Pasteurella multocida	Chickens that received MetII adjuvanted vaccinations (using the Ptfa protein) benefitted from higher protection rate (88%) when challenged. MetII may play an adjuvant role	*Pasteurella multocida*	(76)
OaBac5mini	OaBac5 is a Bac5 homolog derived from sheep neutrophils. (belongs to the cathelicidin family)	RFRPPIR-RPPIRPPFR-PPFRPPVR	Chicken, Challenge with Salmonella Pullorum	The rise in bacterial loads and organ indices was considerably reduced by recombinant OaBac5mini. The colonization of S. Pullorum was suppressed by recombinant OaBac5mini. Through the TLR4/MyD88/NF-B pathway, recombinant OaBac5mini modulated innate immunity to reduce inflammation.	Pullorum disease (PD)	(77)
Mastoparan X (MPX)	Bee venom	H-INWK-GIAAMA-KKLL-NH2	Chicken, MPX (20 mg.kg^{-1}) added to the basal diet.	In addition to lowering the feed conversion ratio, increasing villus length, maintaining normal intestinal architecture, and lowering the expression levels of genes linked to inflammation, MPX also enhanced immune organ index and performance. The tight junction proteins ZO-1, Claudin-1, Occludin, JAM-2, and MUC2 as well as the digestive enzymes FABP2 and SLC2A5/GLUT5 have elevated mRNA expression in response to MPX. MPX increased the Lactobacillus and Lactococcus in the cecum.	Intestinal inflammation	(78)
OH-CATH30	A cathelicidin antimicrobial peptide derived from the king cobra	KFFKKL-KNSVK-KRAKKFFK-KPRVIGVSIPF	Rabbits ocular administrations of AMP solution (1 mg.mL^{-1}).	Significant and noteworthy improvements in clinical symptoms in treatment of bacterial keratitis (*S. aureus*).	Keratitis	(79)
Mastoparan X (MPX)	Bee venom	H-INWKGIAA-MAKKLL-NH2	BALB/c mice, IP injection of MPX (20 mg.kg^{-1}). Challenge with *E. coli*	Decrease the bacterial colonization, reduce intestinal inflammation, effective alleviation of intestinal pathological damage.	Intestinal inflammation	(80)

SET-M33	A non-natural antimicrobial peptide	It consists of four identical short peptides linked to the branched structure (KKIRVRLSA) by a lysine core	BALB/c mice, Intratracheal administration of AMP at 0.5, 2 and 5 mg.kg^{-1}	Considerably reduced the number of BAL neutrophil cells following an LPS exposure. Following SET-M33 delivery, a noteworthy decrease in pro-inflammatory cytokines, including KC, MIP-1_, IP-10, MCP-1, and TNF-α, was also observed.	Pulmonary Inflammation	(81)
SET-M33	A non-natural antimicrobial peptide	It consists of four identical short peptides linked to the branched structure (KKIRVRLSA) by a lysine core	Sprague Dawley rats And beagle dogs. Intravenous injection.	Repeated administration of SET-M33 by short infusion in dogs revealed a no-observed-adverse-effect-level of 0.5 mg/kg/day. But: degeneration and regeneration were found in kidney when SET-M33 was administered at the highest doses.	Toxicity studies	(82)
DPK-060	Human kininogen-derived antimicrobial peptide	GKHKNK-GKKNGKH-NGWKWWW	BALB/c mice,	For the superficial skin wound infection test, (Gel+(DNG[−]+DPK-060)) with 0.5 wt% and 1 wt% peptide exhibited significant ($p < 0.05$) antibacterial effect toward *S. aureus.*	Skin infection	(83)

The recombinant *Pichia pastoris* strains (GS115/Ac-AMP2 and GS115/MiAMP1) that express these peptides have shown promising post-harvest food preservation capabilities in pears infected with the fungus *P. expansum*. Antimicrobial agents such as nisin, pediocin, and mytichitin-CB have been used as food preservatives. Some studies have demonstrated that certain AMPs can inhibit the growth of foodborne pathogens such as Salmonella and E. coli in a variety of food products (49, 51).

9.3. Wound Healing

Several studies suggest that AMPs can aid in wound healing by promoting tissue repair, reducing inflammation, and preventing bacterial colonization at the site of injury (52).

9.4. Cancer Therapy

Chemotherapy medications are frequently used in cancer treatment, but they can have serious adverse effects because they are cytotoxic to both healthy and malignant cells. Some AMPs can bind to and damage cancer cell membranes selectively, leading to their destruction while sparing healthy cells. Cationic amphipathic peptides may be a useful source of anticancer drugs that are selective and impervious to existing resistance mechanisms because of the higher amounts of negatively charged phosphatidylserine on the surface of cancer cells compared to normal cells (53). This selectivity makes AMPs a promising option for reducing the side effects associated with traditional chemotherapy.

10. Examples of AMPs and Recombinant AMPs

Research has shown that recombinant AMPs can be effective in treating a variety of diseases. During an in silico study, Moradi *et al* (54) demonstrated that VcTI peptide from *Veronica hederifolia* can suppress the activity of Papain-like protease (PLpro), a major enzyme in the SARS-CoV-2, by interaction at the active site of PLpro. Therefore, it can be potentially used to halt the replication of SARS-CoV-2. In addition, some peptides, especially HR2-based peptides, have antiviral effects on the coronaviridae family (55).

It has been indicated that the mammary gland tissue-specific vector can be employed for the expression of tracheal antimicrobial peptide (TAP) and treatment of bovine mastitis in mice (56, 57). A neutrophils-derived antimicrobial peptide, rBPI21, interacts preferentially with bacteria's LPS with high affinity and can be used against meningitis (58). Fjell *et al*. (59) found that a synthetic AMP called

LTX-109 was effective in the treatment of skin infections resulting from *Staphylococcus aureus* in dogs. In another study, it was found that hamp1 (QSHLSLCRWCCNCCRGNKGCGFCCKF), or hamp2 (HSSPGGCRFCCNCCPNMSGCGVCCRF) administration, had beneficial effects on reducing iron overload and bacterial loads in fish animal model, respectively (60). Researchers at McMaster University in Canada have developed a cationic antimicrobial peptide called WLBU2 that has been demonstrated to have a great efficacy on both Gram-positive and Gram-negative bacteria, including those that are resistant to commonly used antibiotics. In addition, this peptide has synergistic effects with antibacterial agents against multi-drug-resistant *Acinetobacter baumannii* and *Klebsiella pneumonia* (61). This could suggest that AMPs can used as a novel class of therapeutics for bacterial infections in animals. The results of recent studies on applying antimicrobial peptides in animal models are shown in **Table 1**.

11. Recombinant cLF36, A Promising Antimicrobial Peptide

The most prevalent protein in milk, lactoferrin, has a critical function in a variety of biological processes, such as controlling iron metabolism, immunomodulation, serving as the body's first line of defense against microorganisms, inhibiting lipid peroxidation, and exhibiting antimicrobial activity against a variety of pathogens, including parasites, fungi, bacteria, and viruses (62)". The N-terminal tail of lactoferrin, which contains several peptides such as lactoferricin and lactoferrampin, is the area where the majority of lactoferrin's antibacterial activity is present (47). In a study, camel lactoferrcin (RVKKMRRQWQACKSS) and lactoferrampin (DLIWKLLVKAQEKFGRGKPS) were joined together, and a strong recombinant antimicrobial peptide, named cLF36 (DLIWKLLVKAQEKFGRG KPSKRVKKMRRQWQACKSSHHHHHH) was produced using genetic engineering technology (**Fig. 2**). The antibacterial activity of this novel antimicrobial peptide was evaluated on bacterial plant pathogens, including *Pseudomonas syringae*, *Pseudomonas viridiflava*, *Pseudomonas tolaasii*, *Xanthomonas translucens*, *Xanthomonas oryzae*, *Xanthomonas perforans*, *Erwinia amylovora*, *Pectobacterium carotovorum*, *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes*, *Brenneria nigrifluens*, and *Rhodococcus fascians*. The MIC values were evaluated between 0.39 and 25.07 µg/ml for different bacterial isolates (47). Additionally, the peptide exhibits excellent activity against several clinically isolated avian pathogens, such as *Salmonella enteritidis*, *Streptococcus epidermicus*, *Escherichia coli*, *Pseudomonas aeroginosa*, and *Staphylococcus aureus* (63).

In a different investigation, *Lactococcus lactis*, a significant and food-grade probiotic, was introduced via this broad-spectrum chimeric antimicrobial peptide. Therefore, the probiotic became a cell factory for the production of antimicrobial peptides (38). Through additional in vitro analysis and molecular dynamic stimulation, the security of this product and its mode of action were assessed (64).

Furthermore, it has been found that the substitution and deletion of particular amino acids can significantly improve the peptide's antimicrobial properties (43, 65). Molecular dynamics simulations have been employed to stimulate the peptide's molecular characteristics. In addition, the antiviral activity of this peptide, especially the influence of peptides on hepatitis C virus, avian influenza virus (H5N8 subtype), and bovine rotavirus, has been investigated in several studies (66-69).

In two other studies, it has been observed that the antimicrobial peptide cLF36 has beneficial effects on various aspects of gut health, including intestinal morphology, microflora, junctional proteins, and immune cells in broilers challenged with E. coli and necrotic enteritis (42, 70). Designing novel antimicrobial peptides with more activity and lower toxicity can be accomplished using the novel method known as computational peptide engineering. In addition, the exact molecular interaction of peptides with pathogens can be stimulated *in silico*. This approach has been used successfully in several studies (43, 65)

12. Future of rAMPs

rAMPs are a relatively recent development in the field of biotechnology, and as such, much of the research into their potential applications is still in the preclinical stage. However, despite this early stage of development, there are already some promising results suggesting that rAMPs could have multiple applications in medicine and biotechnology. Recombinant DNA technology can be used to produce rAMPs, enabling their mass

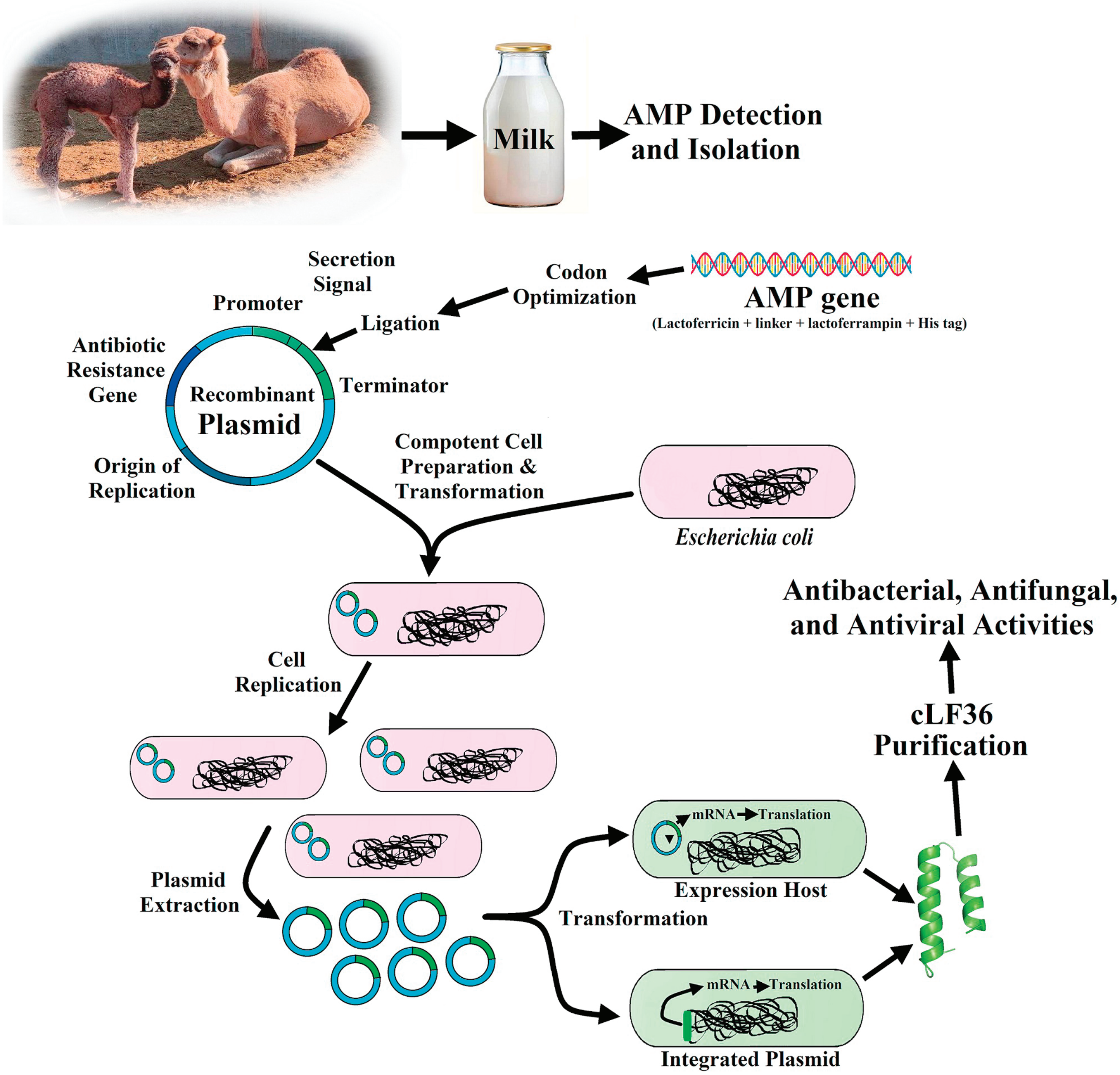

Figure 2. The procedures involved in creating recombinant antimicrobial peptides using genetic engineering technologies. In this image, cLF36 was used as an example. Any natural peptide can be developed using this process.

manufacture and purification (40, 71, 72). Additionally, they have low toxicity, and the risk of developing resistance to them is low.

One of the most important factors that prevented the widespread usage of AMPs in medicine is the high cost of production in large quantities. However, advances in biotechnology and peptide synthesis may help overcome this challenge in the future. The requirement for additional research in order to determine the optimal dose and mode of administration is another challenge. Besides, the effectiveness and safety of various rAMPs, as well as methods to enhance their synthesis and delivery, will need to be meticulously investigated by researchers.

Recombinant DNA technology is one of the most promising methods for the production of AMPs. rAMPs are typically produced using genetic engineering, which involves inserting the gene encoding the AMP into a host organism such as bacteria, yeast, or mammalian cells (**Fig. 2**). The host organism then produces the rAMP, which can be purified and used for therapeutic purposes. In addition, it is possible to produce a transgenic probiotic with AMP genes. In this situation, the probiotic bacteria can produce recombinant AMP in the gastrointestinal tract of patients. Therefore, it can be used as a food or feed additive for improving gut health and treating gastrointestinal infections. One advantage of using rAMPs is that they can be produced in large quantities using standardized manufacturing processes, which can help reduce costs and increase accessibility.

13. Conclusion

Addressing antibiotic resistance requires a multifaceted approach that includes improving infection prevention and control measures, reducing unnecessary antibiotic use, and promoting the innovation of novel antimicrobials and alternative therapies. AMPs are a diverse group of molecules that are produced by a wide range of organisms, including plants, animals, and microorganisms. They have broad-spectrum antimicrobial activity against bacteria, fungi, viruses, and parasites, making them attractive candidates for the treatment of infectious diseases in animals (40, 73). Several studies have investigated the efficacy of AMPs in veterinary medicine, with promising results. The use of recombinant AMPs, produced by genetic engineering, in veterinary medicine has the potential to reduce the use of antibiotics and mitigate the problem of antibiotic resistance. Continued research in this area is needed to determine the safety, efficacy, and cost-effectiveness of rAMPs.

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Competing interests

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Conflict of interests

Authors report no conflict of interests with respect to this work.

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