Antimicrobial Peptides: A Potent Alternative to Antibiotics

Mariam Rima 1, Mohamad Rima 2, Ziad Fajloun 2,3, Jean-Marc Sabatier 4,*, Burkhard Bechinger 5,6 and Thierry Naas 1,7,8,*

1 Team ReSIST, INSERM U1184, School of Medicine Université Paris-Saclay, 94270 Le Kremlin-Bicêtre, France; mariamrima6@gmail.com
2 Laboratory of Applied Biotechnology, Azm Center for Research in Biotechnology and Its Applications, EDST, Lebanese University, Tripoli 1300, Lebanon; mohamad.rima@hotmail.com (M.R.);
ziad.fajloun@ul.edu.lb (Z.F.)
3 Department of Biology, Faculty of Sciences III, Lebanese University, Tripoli 1300, Lebanon
4 Institut de Neuro Physiopathologie, UMR7051, Aix-Marseille Université, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13005 Marseille, France
5 Institut de Chimie de Strasbourg, CNRS, UMR7177, University of Strasbourg, 67008 Strasbourg, France;
bechinge@unistra.fr
6 Institut Universitaire de France (IUF), 75005 Paris, France
7 Bacteriology-Hygiene Unit, Assistance Publique/Hôpitaux de Paris, Bicêtre Hospital, 94270 Le Kremlin-Bicêtre, France
8 French National Reference Centre for Antibiotic Resistance: Carbapenemase-Producing Enterobacterales, 94270 Le Kremlin-Bicêtre, France
* Correspondence: sabatier.jm1@libertysurf.fr (J.-M.S.); thierry.naas@aphp.fr (T.N.);
Tel.: +33-1-45-21-29-86 (T.N.)

Abstract: Antimicrobial peptides constitute one of the most promising alternatives to antibiotics since they could be used to treat bacterial infections, especially those caused by multidrug-resistant pathogens. Many antimicrobial peptides, with various activity spectra and mechanisms of actions, have been described. This review focuses on their use against ESKAPE bacteria, especially in biofilm treatments, their synergistic activity, and their application as prophylactic agents. Limitations and challenges restricting therapeutic applications are highlighted, and solutions for each challenge are evaluated to analyze whether antimicrobial peptides could replace antibiotics in the near future.

Keywords: antimicrobial peptides; antibiotic resistance; multidrug resistance; ESKAPE

1. Introduction

There was collective enthusiasm about the advent of antibiotic therapy, and this optimism prevailed in the light of the constant discoveries of new classes of antibiotics, despite the very early description of therapeutic failures and resistance to treatments with these drugs [1]. At the present, in 2021, multidrug-resistant (MDR) and even pan-drug-resistant (PDR) bacteria have spread widely around the world and are currently responsible for increasing morbidity and mortality rates, as well as the significant cost to society [2]. The low frequency of discoveries of new classes of antibiotics, and the rapid emergence of resistance to novel antibiotics, show the need for novel therapeutic alternatives to antibiotics, such as lysine-based small molecules, vaccines, anti-virulence strategies, phage therapy and antimicrobial peptides.

Antimicrobial resistance (AMR) is an emerging global health problem that results, in some cases, in difficulties to treat bacterial infections. It was listed by the World Health Organization (WHO) among the top ten global public health threats facing humanity, as it is predicted to cause about 10 million deaths each year by 2050 [3]. Therefore, efforts to slow down the propagation of AMR have been implemented worldwide. As such, a Global Action Plan on Antimicrobial Resistance (GAP) was created in 2015, aiming to implement national action plans to limit the progress of AMR. In addition, the WHO reports call
for urgent action to avert an antimicrobial resistance crisis and insists on the importance of discovering and developing new antibiotics. Thus, new compounds that are active against pathogens, especially those which cause nosocomial infections and tend to adopt multidrug resistance, are needed. To curb this problem, several alternative therapies have been proposed, among which antimicrobial peptides (AMPs) were suggested to be very promising more than 20 years ago, as they have existed in nature for millions of years with almost no or limited resistance development [4]. This makes them very attractive compared to antibiotics that develop resistance relatively fast. This absence/slow development of resistance against microbes may be attributed to the presence of various modes of action of AMPs against the bacteria in comparison to the fixed targets used by the antibiotics [5]. In addition, AMPs are considered less toxic, as they are broken down into amino acids unlike other therapeutics, which might generate potentially harmful metabolites. This review is to highlight where these molecules stand now in the overall scheme to curb MDR bacterial infections. Their potential to counteract AMR, to replace traditional antibiotics, to evaluate their benefits and to describe the challenges faced by R&D will be discussed in this review.

AMPs are small polypeptide molecules, typically made up of around 12 to 50 amino acids, found in all classes of living organisms [6]. These molecules are produced as secondary metabolites, are part of the innate immunity and are, in mammals, usually ribosomally produced by epithelial cells, but also by phagocytes (cells of the immune system). These peptides can be found in tissues or mucous membranes; in fact, the latter harbor a multitude of pathogenic or commensal microorganisms. Among these peptides, some have a broad spectrum of antimicrobial activity, capable of inhibiting or killing different types of microorganisms (MO) (Gram-positive or -negative bacteria and/or fungi) but also protozoans, and viruses [7,8]. According to The Antimicrobial Peptide Database, more than 3257 antimicrobial peptides have been described to date [9]. They originate from six kingdoms, where 365 come from bacteria, 5 from archaea, 8 from protists, 22 from fungi, 360 from plants, and 2414 from animals, in addition to some synthetic peptides. Recently, another webserver with several subsections that screen among 40,000 entries has been made available. It summarizes all available data for desired peptide properties, thus allowing further structural and functional studies [10].

2. Classification and Mode of Action of Antimicrobial Peptides

2.1. Classification

AMPs are usually classified according to several criteria: First, based on their biological source. In this classification, we distinguish AMPs from human and mammalian sources, such as cathelicidin and defensin, which make up the most important families of AMPs [11]; AMPs from amphibians [12], fish [13], insects [14], and plants [15] are also classified in this category. Classification could be based on the AMP’s biological functions; for example, antibacterial, antiviral, antifungal, antiparasitic peptides were described. Third, based on their biochemical properties (amino acid sequence, composition, length, hydrophobicity, charge), where conformation and structure serve as criteria for classification as well [7].

2.2. Mode of Action

Some factors can modulate the activity and specificity of AMPs, such as size, charge, hydrophobicity, secondary structure, or amphiphilic character. The conformation of AMPs may also play a role in antimicrobial activity. Indeed, it has been shown that peptides possessing amphipathic structures interact better with the membrane of pathogens. AMPs can have a membrane permeabilization action and/or act on certain intracellular functions (Figure 1) [16].

2.2.1. Membrane Permeabilization

The bacterial membrane is the most important target of AMPs as they act by disrupting the integrity of the pathogen’s membrane [17]. Several models have been described in the literature, all of which result in osmotic lysis. Membrane disruption may happen through
different models, as recently reviewed in Huan et al. [7]. (i) The “barrel-stave” model indicates that peptides insert into the membrane and direct their hydrophobic regions toward the lipid core of the bilayer, forming a transmembrane pore. Experimental evidence for this model has been obtained from structural and biophysical investigations of very hydrophobic sequences, some being devoid of any charge [18]. (ii) The “carpet” model suggests that AMPs accumulate parallel to the membrane surface, forming a “carpet” [19]. Once a threshold concentration of peptides has been reached, they exert a “detergent” effect, leading to the rupture of the cell membrane. Extensive experimental evidence for this mechanism has been obtained for many cationic amphipathic peptides such as magainins [20]. (iii) The “toroidal pore” model implies that AMPs insert themselves perpendicularly into the membrane through interactions between the lipid bilayer and the hydrophilic region of the peptides. In doing so, the membrane distorts and thus forms a “toroidal pore” [21]. (iv) The “aggregate” model assumes the formation of aggregates of peptides and lipids, allowing the translocation of AMPs across membranes. When considering these models, it should be kept in mind that on the one hand, lipid bilayers are soft and can adapt their shapes and thicknesses to the membrane-inserted peptides. On the other hand, the peptides cover a highly dynamic and flexible conformational space and upon interaction with the membrane also respond. Thereby, many different supramolecular arrangements can be formed depending on the lipid composition, peptide concentration, salt, and buffer. These considerations have been incorporated into the SMART model, which takes into consideration that peptides and lipids adjust their conformation and shape when interacting with each other thereby covering a full range of possibilities of interactions between AMPs and membranes [22].

Figure 1. Mechanisms of action of antimicrobial peptides.
2.2.2. Inhibition of Intracellular Functions

Some peptides cross the cytoplasmic membrane and target cellular processes essential for the survival of the pathogen, including inhibition of DNA replication, protein synthesis, interference with nucleic acid biosynthesis, metabolism, cell division, cell wall, and LPS binding proteins [23].

Indeed, some cationic AMPs have been shown to complex and flocculate nucleic acids or other anionic macromolecules due to their affinity for negatively charged phosphodiester bonds [24,25]. For example, Frenatin 2.3S internalizes within bacterial cells after destabilizing their membranes and can bind nucleic acids [26]. Others are able, even at low concentrations, to affect protein synthesis. The examples are countless; for instance, tryptophan-containing AMPs were proven to be efficient at killing *Pseudomonas aeruginosa* by down-regulating the expression of DNA replication-initiating genes [27]. In addition, Tur1A, an AMP found in dolphins, binds to ribosomes, and blocks the translation of mRNA into proteins [28]. Thanatin, an insect-derived AMP targets the bacterial LPS and induces the LPS-mediated aggregation [29]. Tridecaptin blocks ATP synthesis in bacteria and is active on MDR and colistin-resistant Enterobacterales [30].

2.2.3. Immunomodulatory Activity

Well characterized for their antimicrobial activities, AMPs are also known for their immunoregulatory functions. They can thus participate in the recruitment and activation of immune cells.

It has been described in the literature that certain AMPs, such as defensins, can enhance the production of inflammatory cytokines, such as interleukin-1 [31]. To mention another example, cathelicidin BF has been shown to exhibit immunomodulatory activity that, in mice, is able to ameliorate pneumonia caused by *Pseudomonas aeruginosa* [32].

2.2.4. Action on Biofilms

When in the form of biofilms, pathogens escape more easily the action of conventional antimicrobials. Indeed, microorganisms in biofilms are capable of tolerating, in particular, *via* transcriptional regulatory mechanisms, high concentrations of antimicrobials even though they are totally sensitive in planktonic conditions [33].

The mode of action of AMPs on biofilms is not yet fully understood; however, it is recognized that they can prevent different stages of biofilm formation. It has been shown that some AMPs can inhibit the adhesion of bacteria to surfaces by reducing certain types of motilities such as “swarming” or “swimming”. They are also able to stimulate “twitching”, another type of motility known to promote the disassembly of biofilms. AMPs can also down-regulate certain genes involved in quorum sensing, the latter being known to play a role in biofilm formation and/or in the organization and communication of bacteria within the biofilm [34]. Thus, it has been demonstrated that LL-37 cathelicidin targets quorum sensing mechanisms that control *P. aeruginosa* biofilm formation [35].

3. AMPs as Potential Alternatives to Antibiotics

Several advantages were observed for AMPs over classical antibiotics: (i) usual resistance mechanisms observed toward conventional antibiotics are bypassed by AMPs [36,37]; (ii) they are easier to synthesize since they consist usually of short amino acid sequences [38]; (iii) they show rapid killing [39]; (iv) they act on bacteria irrespective of their resistance phenotype, since they are not affected by the known resistance mechanisms [40–42], and (v) they do not affect microbiota, which are often disrupted by conventional antibiotics [43]. These advantages made it possible to have several AMPs that are now in medical use, such as nisin, gramicidin, polymyxins, daptomycin, and melittin (reviewed by Dijksteel et al. [44]).
3.1. Activity against MDR Bacteria and ESKAPE

Hundreds of AMPs have shown antibacterial activity against resistant bacteria in vivo [45]. Among the most recent examples, a novel piscidin-like peptide called cerocin from black sea bass fish was described to exert broad-spectrum antimicrobial activity against several bacteria, especially Gram-positive pathogens [46]. Similarly, jelleine-I, a small AMP formed by eight amino acids, acts against both Gram-negative and Gram-positive bacteria, mainly by disrupting the integrity of the cell membrane [47]. Furthermore, another novel defensin-like peptide was described recently for its antimicrobial activity against Gram-positive bacteria, including Staphylococcus aureus, Staphylococcus carnosus, Nocardia asteroides, and one tested Gram-negative bacterium Psychrobacter faecalis [48]. Interestingly, AMPs can act against bacteria of the ESKAPE complex, which is made up of six pathogens responsible for most of the nosocomial life-threatening infections: Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa, and Enterobacter spp. [49]. ESKAPE pathogens are usually MDR, which limits therapeutic options and puts patients’ lives at risk. Many AMPs showed promising activity against ESKAPE pathogens. For example, bip-P-113, dip-P-113 and nal-P-133 are three derivatives of the AMP p-133, that work well against E. faecium, showing a low MIC of 4 μg/mL as compared to 64 μg/mL for vancomycin [50].

The resistance of S. aureus to methicillin remains a huge drawback associated with high mortality rates [51]. Many AMPs are able to eradicate methicillin-resistant S. aureus (MRSA) [52]; for example, poly(2-oxazoline)s, an easy-to-synthesize polymer mimetic of AMPs that displays high and selective antimicrobial activity against MRSA, is considered as a promising antimicrobial candidate due to its low MIC values (12.5 μg/mL) [53]. AM3 is a novel synthetic peptide consisting of a short amino acid sequence that has low toxicity coupled to a great biological activity, especially against S. aureus strains. The low MICs of AM3 (5 μg/mL) against S. aureus (ATCC25923) and MRSA (7.5 μg/mL) [54], make it a great AMP candidate. Finally, SAAP-148, a derivative of the human LL37 peptide, is active against several resistant ESKAPE pathogens (S. aureus, P. aeruginosa, A. baumannii), including biofilms that occur in wound infections [55].

K. pneumoniae, is a challenging bacterium with respect to conventional antibacterial therapy, as it may be encapsulated, limiting the antibiotic’s capacity to penetrate. However, this bacterium is sensitive to pepW, an AMP that targets, aggregates and disrupts K. pneumoniae’s capsules with low MICs (2–4 μg/mL) [56]. PepW is also active against Escherichia coli with even lower MICs of 1–2 μg/mL [56]. Similarly, AA139 and SET-M33 may be promising novel drugs against MDR K. pneumoniae strains with MICs ranging from 4 to 16 μg/mL, but more interestingly, they remained active even on colistin-resistant K. pneumoniae isolates [57].

Aurein 1.2, CAMEL, citropin 1.1, LL-37, Cec4 and omiganan have shown excellent activity against A. baumannii, with MICs going from 2 to 16 μg/mL [58,59]. Consequently, AMPs are considered as potential candidates for new treatments since they have relatively low MICs against a bacterial strain for which only limited, if any, treatment options are available. As reviewed by Neshani et al., 46 AMPs exhibit good activity against A. baumannii, some of which could be used as preventive and others as therapeutic options [60]. DN4 and DC4 have shown significant activity against A. baumannii as well as in a K. pneumoniae model, where an MIC of 32 μg/mL was noted [61].

On the other hand, ZY4 permeabilizes the MDR P. aeruginosa membrane and exhibits killing with MICs ranging from 2 to 4.5 μg/mL [62]. GL13K was also described as an active AMP showing good activity against MDR P. aeruginosa, with an MIC of 8 μg/mL [63,64]. Furthermore, NCK-10 is a small molecule mimetic made of a naphthalene aromatic ring, a decyl chain and a cationic lysine with potent P. aeruginosa antibiofilm activity [65].

3.2. Synergistic AMPs

To date, and according to the AMPs database, 47 of the listed AMPs show synergistic activities against several pathogens. This synergistic/additive effect is of interest to find
new efficient therapeutic strategies against the ESKAPE pathogens [66]. Synergies may be observed between different AMPs, AMPs of the same family or AMPs and antibiotics. Table 1 summarizes a few examples of AMPs that showed good synergistic effects.

### Table 1. Properties of AMPs showing synergistic activities with one another and/or with antibiotics.

| AMPs      | Source                  | Synergistic Molecule | Target                              | Refs.   |
|-----------|-------------------------|----------------------|-------------------------------------|---------|
| PGLa      | Frog skin               | Magainin 2           | E. coli and S. aureus               | [67]    |
| Ranalexin | -Bullfrog R. catesbeiana -Staphylococcus simulans | Endopeptidase lysostaphin | S. aureus (MRSA)                   | [68]    |
| Tridecaptin M | Mud bacterium | Rifampicin, vancomycin, and ceftazidime | Extremely drug-resistant A. baumannii | [69]    |
| Dermaseptin | Amphibians skin | Dermaseptin | E. coli, P. aeruginosa, S. aureus | [70,71] |
| Bactenecin | Lactic acid bacteria | Bactenecin | E. coli, P. aeruginosa, S. Typhimurium | [72,73] |
| Lactoferricin | Mammalians | Ciprofloxacin, ceftazidime | P. aeruginosa | [74]    |
| Nisin     | Lactococcus lactis      | Colistin              | Pseudomonas biofilms                | [75,76] |
| P10       | Ceftazidim/ doripenem   | MDR A. baumannii and colistin-resistant P. aeruginosa | [76]    |
| Gad-1     | Fish                    | Kanamycin, ciprofloxacin | P. aeruginosa | [77]    |

### 3.3. AMPs Role in Preventive Healthcare

#### 3.3.1. Prophylaxis

Another potential domain of action of AMPs is preventive healthcare, which deals with the prevention of illness [78]. As such, polycationic peptides were used as prophylactic agents against methicillin-susceptible or methicillin-resistant Staphylococcus epidermidis [79]. Antibacterial peptide-based gels were also synthesized to prevent medical implanted device infections [80]. Likewise, coating antimicrobial peptides was used for the prevention of urinary catheter-associated infections [81]. For example, bactericidal activity of chain 201D, an AMP derived from crowberry endophytes, was effective against E. coli and S. aureus, when immobilized on a model surface where it can bind and kill, by contact, a high percentage of adherent bacteria [81]. This gives AMPs a high potential for the development of antimicrobial surfaces, namely for application in urinary catheters [81]. Another example of AMP-based infection prevention in healthcare is dental caries caused by several pathogens [82]. Some AMPs including α-helical ones prevent bacterial adherence to implant surfaces [83].

#### 3.3.2. Biofilm Treatment

Biofilms are one of the most common spreaders of infectious disease [84]. They form when communities of microorganisms adhere to some surfaces, and they are usually hard to treat [85]. Biofilms can develop on catheters, breast implants, or even on living tissue such as lungs or teeth, putting patients at risk of developing nosocomial infections that are difficult to eradicate with antibiotics [86]. By living in biofilms, bacteria are protected from harmful conditions, and thus can develop resistance to common antibiotics, which makes AMPs promising alternatives for new therapeutic strategies [87,88]. In this context,
as already reviewed by Galdiero et al., many AMPs serve as agents that interfere with bacterial biofilm formation/expansion [89], inhibit bacterial adherence on indwelling medical devices, and therefore can reduce nosocomial infections [81,90]. For example, vancomycin-coated tympanostomy tubes resist MRSA biofilm formation [91]. Interestingly, another AMP of the cathelicidins family D-LL-31 greatly enhances the biofilm-eradicating effects of currently used antibiotics [92,93]. Furthermore, SAAP-148, a synthetic AMP, showed great antibiofilm activity, where it eradicates MRSA and MDR A. baumannii [55]. Similarly, KKd-11 is a remarkable AMP that was not only able to inhibit the formation of biofilms but also effectively kill the bacteria within the biofilms [94]. Together, these findings show interesting potential of AMPs, which is a step forward as compared to conventional antibiotic therapy.

3.3.3. Vaccination: AMP-Based Vaccines

In the context of the preventive healthcare field, AMPs could also be used as vaccines or vaccine adjuvants to induce protective immunity or immune response against infections [95]. As such, IC31, a synthetic adjuvant containing the AMP KLKL5KLK, can induce a protective immune response against the desired antigen [96]. It was also proven to be a potent adjuvant to a DNA vaccine targeting Mycobacterium tuberculosis [95]. However, no vaccine exists until today against MDR bacterial isolates [97]. Therefore, in this present scenario of exponentially increasing AMR, using the immuno-modulatory advantages of AMPs could help in alleviating AMR problem. The development of AMP-based vaccines may be able to reduce the burden and to overcome the AMR issue worldwide [98].

4. AMPs for Diagnostic Purposes

Rapid diagnostic tools are the key to prevent the spread of contagious infectious agents by rapidly identifying carriers or infected patients and prompt implementation of adapted infection control measures. The ability of AMPs to sense bacterial presence in potentially infected clinical samples is a major advantage. As already reviewed by Pardoux et al., AMPs could serve as infection selective tracers where they act as probes in biosensors and thus detect pathogens [99]. This is due to the preferential binding of AMPs along with their great number allowing the coverage of a wide range of microorganisms. AMPs were also described as powerful radiotracers and proved to be even better than labeled antibiotics [100]. [99mTc-HYNIC/EDDA]-MccJ25 is an example of an antimicrobial peptide analog that was used as a potential radiotracer for detection of infection [99]. Interestingly, this imaging agent for E. coli infection is also stable against enzymatic and metabolic degradation [101].

5. AMPs Facing 2019 Pandemic: SARS-CoV-2

In addition to activity toward bacteria, according to the database of antimicrobial activity and structure of peptides (DBAASP) [102], 208 AMPs have been reported to show antiviral activities against SARS-CoV-2. Slight changes to glycocin F and lactococcin G increased their efficacy against SARS-CoV-2 by inhibiting protein synthesis with no side effects [103]. Similarly, mucroporin-M1 (LFRLIKSLIKRLVSAFK), a peptide analog displaying four amino acid changes (G3R, P6K, G10K, and G11R) as compared to the parent peptide mucroporin shows therapeutic activities against SARS-CoV-2 [104]. Brilacidin and LF are additional drug candidates for SARS-CoV-2 treatment [105,106]. Caerin 1.6 and caerin 1.10, two other AMPs, have a very high potential to interact with the spike surface protein of SARS-CoV-2, but low affinity for the ACE2 receptor. The selectivity of these peptides toward viral proteins makes them potential candidates for SARS-CoV-2 therapy [107].

6. Limitations and Challenges of the Therapeutic Use of AMPs

Despite the numerous advantages of AMPs, several problems prevented them from being used as therapeutic agents in human clinics. For instance, one major problem is their
limited stability, especially their susceptibility to the degradation by proteases [108,109]. In fact, the instability toward proteases hampers the development of many AMPs [110], by limiting their use for topical applications and/or requiring sequence modifications, to make them less susceptible to degradation [111]. Other problems include high extraction costs [112,113], poor bioavailability [6], short half-lives [114], cytotoxicity, and lack of specificity [115,116]. In addition, AMPs tend to display lower direct antimicrobial activity as compared to antibiotics. In real, regulatory agencies want AMPs to exhibit similar or even stronger activities than antibiotics [37]. Finally, resistance development by the bacteria toward AMPs cannot be excluded, especially if the microorganism is often exposed to them [117]. So far, only a few resistance mechanisms to AMPs have been described, including sequestration by bacterial enzymes and export by efflux pumps [118]. Taken together, these limitations slow down the successful reach of AMPs into clinical phases and limit their use to a small fraction of their potential (Figure 2) [44].

![Figure 2. Limitations of AMPs and possible solutions for their therapeutic use.](image)

### 6.1. Clinical Trials of AMPs

Although a huge number of AMPs have been described, very few reach the market. For example, ghrelin, the endogenous appetite peptide, possessing a good antimicrobial activity against Gram-negative bacteria is still in phase II clinical trial [119,120]. Surotomycin, is another example of AMP that is currently under investigation in phase III clinical trials; it targets *Clostridium difficile* and treats the associated diarrhea. The examples are countless; many AMPs remain under investigation until their efficacy is confirmed and secondary effects are eliminated. Some AMPs fail to reach the last clinical trials, such as (i) frulimicin B, which was terminated after phase I clinical trial due to unfavorable pharmacokinetics [44] and (ii) murepavadin, which was eliminated after a phase III clinical trial since it causes acute kidney injuries [44].

### 6.2. How to Overcome Some of These Limitations?

Different strategies were explored to overcome the drawbacks described previously. Modifications such as encapsulation of AMPs could be the key to avoiding their degradation by proteases and thus improving stability [121,122]. Several AMPs have been tested before and after encapsulation, and an obvious enhancement was observed: encapsulation of the AMP IK8, with gold nanorods into polyethylene glycol hydrogels protected the AMP from proteolysis and provided a triggered release of the therapeutic molecule [123]. High extraction costs could be limited by synthesizing AMPs in the lab using natural ones as templates where, at the same time, synthetic AMPs are always an enhanced version of the natural ones [124]. For this, genetic programming could be used to save not only money, but also on time and workload, and this could be started in silico through programs that predict the activity of AMPs before even generating them to avoid unnecessary synthetic efforts [125]. To improve bioavailability, increased half-lives of AMPs, and reduced cytotoxicity, structural modifications such as dimerization [126], methylation and
Antibiotics 2021, 10, 1095

more have been successfully tested [127]. Specificity could be introduced by strategies for site specific release [128], such as specifically targeting AMPs (STAMP), which showed high efficiency in delivering the AMP to the target without harming the natural microbiota [129,130]. This strategy aims to implant AMPs into a drug delivery vehicle composed of the AMP itself in addition to a targeting peptide that allows a specific binding to the desired pathogen [43,129].

6.3. Synthetic AMPs

For decades, research for AMPs was not always successful. As mentioned previously, some AMPs were not introduced into clinics due to their toxicity/ degradation, while others were not very performant. However, none of these efforts were lost as these AMPs played the role of templates for the development of more efficient agents. For example, the introduction of L-phenylalanine into protonectin enhanced its selective antibacterial activity against several Gram-positive bacteria and reduced MIC in S. aureus from 16 to 4 µg/mL [131]. In addition, the cathelicidin-derived antimicrobial peptide PMA-23 was further modified by amino acid substitutions to obtain PMA-23RI (for, Leu5-Arg and Thr19-Ile). The variant exhibited a higher stability and improved antibacterial activity when compared with the original PMA-23, with MICs reduced by half in some cases from a simple modification to the original AMP [132]. Analogs of mastoparan-C were also designed by amino acid substitution and peptide truncation, which reduced cell toxicity and improved the bioactivity of the original AMP [127,133].

Table 2 provides additional information on many AMPs discussed in this paper.

Table 2. Amino acid sequences, type of synthesis and target groups of the AMPs discussed in this paper. Data were retrieved from the database of antimicrobial activity and structure of peptides [102]. GP: Gram-positive bacteria; GN: Gram-negative bacteria.

| AMP           | Amino Acid Sequence | Synthesis | Target Group                          |
|---------------|---------------------|-----------|---------------------------------------|
| Cathelicidin-BF | KFFRLKSSVKKRAKEFFKKPRVYGVSIPF | Ribosomal | GP and GN                             |
| Defensin      | GFGCPDQMCHCHQHTGGRGCGSGPIKLTCTCYR | Ribosomal | GP and GN                             |
| Nisin-P       | VxxKXXpXGXXGXXAxxXGXXHF | Ribosomal | GP and GN                             |
| Gramicidin-S  | VXLPVXLP             | Nonribosomal | GP, GN, parasites, fungus, cancer cells, and mammalian cells |
| Polymyxin     | XTXXXILXT            | Synthetic | GN                                    |
| Mellitin      | GIGAVLWLPLAILRKRQQ   | Synthetic | GP, GN, and mammalian cells           |
| Sm-Piscidin   | KGRQQAKWYKRNMQMNQGQGQG | Ribosomal | GP, GN, and fungus                    |
| Jellein-1     | PFKISHL              | Ribosomal | GP, GN, and mammalian cells           |
| Aurein-1.1    | GLFDHIKKIAESI        | Ribosomal | GP and GN                             |
| Citropin-1.1  | GLFDVKKVASVIGGL      | Ribosomal | GP, GN, fungus, mammalian cells       |
| Omiganan      | ILRWPWWVPWRK         | Synthetic | GP, GN, fungus, and mammalian cells   |
| ZY4           | VCKRWKKWKWRKWKWCV     | Synthetic | GP, GN, fungus, and mammalian cells   |
| GL33K         | GKIILKASLKL          | Synthetic | GP, GN, insects, and mammalian cells  |
| Chain201D     | KIWIRRFFRKR          | Synthetic | GP, GN, and fungus                    |
| SAAP-148      | LKRRWKRFRFKLWRRWQRLKPV | Synthetic | GP and GN                             |
| Lactococcin-G | GTWDDICQGCRAYWVCAKCMNNSYDQNASRINRKHKKHWWGWLAWVPAGEFLKFGGKAIEGKNKDKWKNI | Ribosomal | GP                                    |
| Caerulin-1.10 | GLLSSLVVSXKHLVPHVPVLAEKL | Ribosomal | GP, GN, viruses, and mammalian cells  |
| Murepavadin   | LSYXXXXWXASPP        | Synthetic | GP, GN, cancer cells, and mammalian cells |
| Protonectin   | ILGTLPLKXGL         | Ribosomal | GP, GN, cancer cells, fungus, and mammalian cells |
7. Conclusions

AMPs constitute promising agents with good antibacterial activity against a large set of pathogens, including Gram-negative and Gram-positive bacteria. Their ability to kill ESKAPE pathogens, especially those being pan-drug-resistant, represents a clinical added value over antibiotics due to the limited treatment options available to eradicate such infections. In this review, examples of AMPs effective against MDR bacteria that have the potential to replace conventional antibiotics have been reported. Their roles in boosting the activity of therapeutic molecules, eradication of biofilms, and preventive healthcare broaden AMPs’ therapeutic potential (Figure 3). However, despite the huge number of AMPs described, and the efforts to optimize them, only a few reached advanced clinical trials. There are several challenges, such as their low stability, high extraction costs, and cytotoxicity, which need to be overcome so AMPs can be used in clinics. Nevertheless, due to increasing research efforts, it is expected that, hopefully, potent alternatives to antibacterial agents with the ability to eradicate untreatable life-threatening infections will be developed soon.

![Figure 3. Potential use of AMPs.](image)

**Author Contributions:** Conceptualization, M.R. (Mariam Rima), M.R. (Mohamad Rima), and T.N.; literature search, M.R. (Mariam Rima), M.R. (Mohamad Rima), and J.-M.S.; validation of search results, M.R. (Mariam Rima), M.R. (Mohamad Rima), Z.F., B.B., J.-M.S. and T.N.; writing—original draft preparation, M.R. (Mariam Rima), M.R. (Mohamad Rima), T.N. and B.B.; writing—review and editing, all. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Assistance Publique-Hôpitaux de Paris, the University Paris-Saclay, the Institut National de la Santé et de la Recherche Médicale (INSERM), the Laboratory of Excellence in Research on Medication and Innovative Therapeutics (LERMIT) through a grant from the French National Research Agency (ANR-10-LABX-33) and by the ANR-BMBF French-German bilateral project Natural-Arsenal “New Antibiotics Tackling mUlti-Resistance by acting on Alternative bacterial tARgets in ynergy with mEmbrane-disruptiNg AntimicrobialL peptides” (ANR-19-AMRB-0004).

**Acknowledgments:** We are grateful to N. D’Amelio for the helpful advice.

**Conflicts of Interest:** The authors declare no conflict of interest.
References

1. Gots, J.S. Production of extracellular penicillin-inactivating substances associated with penicillin resistance in *Staphylococcus aureus*. Proceedings of the Society for Experimental Biology and Medicine. *Soc. Exp. Biol. Med.* 1945, 60, 165–168. [CrossRef]

2. Friedman, N.D.; Temkin, E.; Carmeli, Y. The negative impact of antibiotic resistance. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. *Clin. Microbiol. Infect.* 2016, 22, 416–422. [CrossRef] [PubMed]

3. O’Neill, J. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations; Government of the United Kingdom: London, UK, 2016.

4. Yu, G.; Baeder, D.Y.; Regoes, R.R.; Rolff, J. Predicting drug resistance evolution: Insights from antimicrobial peptides and antibiotics. *Proc. R. Soc. B Biol. Sci.* 2018, 285, 20172687. [CrossRef] [PubMed]

5. Nicolás, P. Multifunctional host defense peptides: Intracellular-targeting antimicrobial peptides. *FEBS J.* 2009, 276, 6483–6496. [CrossRef] [PubMed]

6. Mahlapuu, M.; Håkansson, J.; Ringstad, L.; Björn, C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. *Front. Cell. Infect. Microbiol.* 2016, 6, 194. [CrossRef] [PubMed]

7. Huan, Y.; Kong, Q.; Mou, H.; Yi, H. Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. *Front. Microbiol.* 2020, 11, 582779. [CrossRef] [PubMed]

8. Wang, E.B.G. Antimicrobial Peptide Discovery, Design and Novel Therapeutic Strategies, 2nd ed.; University of Nebraska Medical Center: Omaha, NE, USA, 2017.

9. The Antimicrobial Peptide Database. Available online: https://wagapd3.com/ (accessed on 1 July 2021).

10. Ramos-Martín, F.; Annaval, T.; Buchoux, S.; Sarazin, C.; D’Amelio, N. ADAPTABLE: A comprehensive web platform of antimicrobial peptides tailored to the user’s activity. *Life Sci. Alliance* 2019, 2, e201900512. [CrossRef]

11. Kościuczuk, E.M.; Lisowski, P.; Jarczak, J.; Strzałkowska, N.; Jóźwik, A.; Horbańczuk, J.; Krzyżewski, J.; Zwierzchowski, L.; Bagnicka, E. Cathelicidins: Family of antimicrobial peptides. A review. *Mol. Biol. Rep.* 2012, 39, 10957–10970. [CrossRef]

12. Patocka, J.; Nepovimova, E.; Klímová, B.; Wu, Q.; Kuca, K. Antimicrobial Peptides: Amphibian Host Defense Peptides. *Curr. Med. Chem.* 2018, 25, 5924–5946. [CrossRef]

13. Masso-Silva, J.A.; Diamond, G. Antimicrobial peptides from fish. *Pharmaceuticals* 2014, 7, 265–310. [CrossRef]

14. Wu, Q.; Patocka, J.; Kuča, K. Insect Antimicrobial Peptides, a Mini Review. *Toxins* 2018, 10, 461. [CrossRef] [PubMed]

15. Tam, J.P.; Wang, S.; Wong, K.H.; Tan, W.L. Antimicrobial Peptides from Plants. *Pharmaceuticals* 2015, 8, 711–757. [CrossRef] [PubMed]

16. Le, J.; Sun, L.; Huang, S.; Zhu, C.; Li, P.; He, J.; Mackey, V.; Coy, D.H.; He, Q. The antimicrobial peptides and their potential clinical applications. *Am. J. Transl. Res.* 2019, 11, 3919–3931. [PubMed]

17. Kumar, P.; Kizhakkedathu, J.N.; Straus, S.K. Antimicrobial Peptides: Diversity, Mechanism of Action and Strategies to Improve the Activity and Biocompatibility In Vivo. *Biomolecules* 2018, 8, 4. [CrossRef]

18. Salnikov, E.S.; Raya, J.; Strazalkowska, N.; Jóźwik, A.; Horbańczuk, J.; Krzyżewski, J.; Zwierzchowski, L.; Bagnicka, E. Cathelicidins: Family of antimicrobial peptides. A review. *Mol. Biol. Rep.* 2012, 39, 10957–10970. [CrossRef]

19. Shai, Y.; Oren, Z. From “carpet” mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides. *Peptides* 2001, 22, 1629–1641. [CrossRef]

20. Aisenbrey, C.; Marquette, A.; Bechinger, B. The mechanisms of action of cationic antimicrobial peptides refined by novel concepts from biophysical investigations. In *Antimicrobial Peptides: Basics for Clinical Application*; Matsuizaki, K., Ed.; Springer: Singapore, 2019; pp. 33–64.

21. Wimley, W.C. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem. Biol.* 2010, 5, 905–917. [CrossRef] [PubMed]

22. Bechinger, B. The SMART model: Soft Membranes Adapt and Respond, also Transiently, in the presence of antimicrobial peptides. *J. Pept. Sci.* 2015, 21, 346–355. [CrossRef]

23. Le, C.-F.; Fang, C.-M.; Sekaran, S.D. Intracellular Targeting Mechanisms by Antimicrobial Peptides. *Antimicrob. Agents Chemother.* 2017, 61, e02340-16. [CrossRef] [PubMed]

24. Moulay, G.; Leborgne, C.; Mason, A.; Aisenbrey, C.; Kichler, A.; Bechinger, B. Histidine-rich designer peptides of the LAH4 family promote cell delivery of a multitude of cargo. *J. Pept. Sci. Off. Publ. Eur. Pept. Soc.* 2017, 23, 320–328. [CrossRef] [PubMed]

25. Shai, Y.; Oren, Z. From “carpet” mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides. *Peptides* 2001, 22, 1629–1641. [CrossRef]

26. Salnikov, E.S.; Raya, J.; De Zotti, M.; Zaitseva, E.; Peggion, C.; Ballano, G.; Toniolo, C.; Raap, J.; Bechinger, B. Alamethicin Supramolecular Organization in Lipid Membranes from (19)F Solid-State NMR. *Biophys. J.* 2016, 111, 2450–2459. [CrossRef]

27. Shai, Y.; Oren, Z. From “carpet” mechanism to de-novo designed diastereomeric cell-selective antimicrobial peptides. *Peptides* 2001, 22, 1629–1641. [CrossRef]

28. Aisenbrey, C.; Marquette, A.; Bechinger, B. The mechanisms of action of cationic antimicrobial peptides refined by novel concepts from biophysical investigations. In *Antimicrobial Peptides: Basics for Clinical Application*; Matsuizaki, K., Ed.; Springer: Singapore, 2019; pp. 33–64.

29. Wimley, W.C. Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem. Biol.* 2010, 5, 905–917. [CrossRef] [PubMed]

30. Bechinger, B. The SMART model: Soft Membranes Adapt and Respond, also Transiently, in the presence of antimicrobial peptides. *J. Pept. Sci.* 2015, 21, 346–355. [CrossRef]

31. Le, C.-F.; Fang, C.-M.; Sekaran, S.D. Intracellular Targeting Mechanisms by Antimicrobial Peptides. *Antimicrob. Agents Chemother.* 2017, 61, e02340-16. [CrossRef] [PubMed]

32. Moulay, G.; Leborgne, C.; Mason, A.J.; Aisenbrey, C.; Kichler, A.; Bechinger, B. Histidine-rich designer peptides of the LAH4 family promote cell delivery of a multitude of cargo. *J. Pept. Sci. Off. Publ. Eur. Pept. Soc.* 2017, 23, 320–328. [CrossRef] [PubMed]

33. Chongsiriratana, N.P.; Lin, J.S.; Kapoor, R.; Wetzler, M.; Rea, J.A.C.; Didwania, M.K.; Contag, C.H.; Barron, A.E. Intracellular biomass flocculation as a key mechanism of rapid bacterial killing by cationic, amphipathic antimicrobial peptides and peptides. *Sci. Rep.* 2017, 7, 16718. [CrossRef] [PubMed]

34. Muñoz-Camargo, C.; Salazar, V.A.; Barrero-Guevara, L.; Camargo, S.; Mosquera, A.; Groot, H.; Boix, E. Unveiling the Multifaceted Mechanisms of Antibacterial Activity of Buforin II and Frenatin 2.3S Peptides from Skin Micro-Organs of the Orinoco Lime Treefrog (*Sphaenorhynchus lacteus*). *Int. J. Mol. Sci.* 2018, 19, 2170. [CrossRef]

35. Han, X.; Kou, Z.; Jiang, F.; Sun, X.; Shang, D. Interactions of Designed Trp-Containing Antimicrobial Peptides with DNA of Multidrug-Resistant Pseudomonas aeruginosa. *DNA Cell Biol.* 2021, 40, 414–424. [CrossRef] [PubMed]

36. Mardirosian, M.; Perébashkine, N.; Benincasa, M.; Gambato, S.; Hofmann, S.; Huter, P.; Müller, C.; Hilpert, K.; Innis, C.A.; Tossi, A.; et al. The Dolphin Proline-Rich Antimicrobial Peptide Tur1A Inhibits Protein Synthesis by Targeting the Bacterial Ribosome. *Cell Chem. Biol.* 2018, 25, 530–539. [CrossRef]
29. Dash, R.; Bhattacharjya, S. Thanatin: An Emerging Host Defense Antimicrobial Peptide with Multiple Modes of Action. Int. J. Mol. Sci. 2021, 22, 1522. [CrossRef] [PubMed]

30. Jangra, M.; Kaur, M.; Tambat, R.; Rana, R.; Maurya, S.K.; Khatri, N.; Ghafur, A.; Nandanwar, H. Tridecaptin M, a New Variant Discovered in Mud Bacterium, Shows Activity against Colistin- and Extremely Drug-Resistant Enterobacteriaceae. Antimicrob. Agents Chemother. 2019, 63, e00338-19. [CrossRef]

31. Steinstraesser, L.; Kraneburg, U.; Jacobsen, F.; Al-Benna, S. Host defense peptides and their antimicrobial-immunomodulatory duality. Immunobiology 2011, 216, 322–333. [CrossRef]

32. Liu, C.; Qi, J.; Shan, B.; Gao, R.; Gao, F.; Xie, H.; Yuan, M.; Liu, H.; Jin, S.; Wu, F.; et al. Pretreatment with cathelicidin-BF ameliorates Pseudomonas aeruginosa pneumonia in mice by enhancing NETosis and the autophagy of recruited neutrophils and macrophages. Int. Immunopharmacol. 2018, 55, 382–391. [CrossRef]

33. Lebeaux, D.; Ghigo, J.M.; Beloïn, C. Biofilm-related infections: Bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol. Mol. Biol. Rev. 2014, 78, 510–543. [CrossRef]

34. de la Fuente-Núñez, C.; Korolik, V.; Bains, M.; Nguyen, U.; Breidenstein, E.B.; Horsman, S.; Lewenza, S.; Burrows, L.; Hancock, R.E. Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. Antimicrob. Agents Chemother. 2012, 56, 2696–2704. [CrossRef] [PubMed]

35. Overhage, J.; Campisano, A.; Bains, M.; Torfs, E.C.W.; Rehm, B.H.A.; Hancock, R.E.W. Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect. Immun. 2008, 76, 4176–4182. [CrossRef] [PubMed]

36. Peschel, A.; Sahl, H.G. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nat. Rev. Microbiol. 2006, 4, 529–536. [CrossRef] [PubMed]

37. Wang, S.; Zeng, X.; Yang, Q.; Qiao, S. Antimicrobial Peptides as Potential Alternatives to Antibiotics in Food Animal Industry. Int. J. Mol. Sci. 2016, 17, 603. [CrossRef]

38. Hansen, I.K.O.; Lovdahl, T.; Simonovic, D.; Hansen, K.O.; Andersen, A.J.C.; Devold, H.; Richard, C.S.M.; Andersen, J.H.; Strom, M.B.; Haug, T. Antimicrobial Activity of Small Synthetic Peptides Based on the Marine Peptide Turgencin A: Prediction of Antimicrobial Peptide Sequences in a Natural Peptide and Strategy for Optimization of Potency. Int. J. Mol. Sci. 2020, 21, 460. [CrossRef]

39. Raheem, N.; Straus, S.K. Mechanisms of Action for Antimicrobial Peptides with Antibacterial and Antibiofilm Functions. Front. Microbiol. 2019, 10, 2866. [CrossRef]

40. Park, S.-C.; Park, Y.; Hahm, K.-S. The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. Int. J. Mol. Sci. 2011, 12, 5971–5992. [CrossRef]

41. Pirtskhalava, M.; Armstrong, A.A.; Grigolava, M.; Chubinidze, M.; Alimbarashvili, E.; Vishnepolsky, B.; Gabrielian, A.; Rosenthal, A.; Hunt, D.E.; Tartakovsky, M. DBAASP v3: Database of antimicrobial/cytotoxic activity and structure of peptides as a resource for development of new therapeutics. Nucleic Acids Res. 2020, 49, D288–D297. [CrossRef]

42. Park, S.-C.; Park, Y.; Hahm, K.-S. The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. Int. J. Mol. Sci. 2011, 12, 5971–5992. [CrossRef]

43. Lee, H.-R.; You, D.-G.; Kim, H.K.; Sohn, J.W.; Kim, M.J.; Park, J.K.; Lee, G.Y.; Yoo, Y.D. Romo1-Derived Antimicrobial Peptide Is a New Antimicrobial Agent against Multidrug-Resistant Bacteria in a Murine Model of Sepsis. mBio 2020, 11, e03528-19. [CrossRef]

44. Li, F.; Gao, Z.; Wang, K.; Zhao, Y.; Zhao, M.; Zhao, Y.; Bai, L.; Yu, Z.; Yang, X. A novel defensin-like peptide contributing to antimicrobial and antioxidant capacity of the tick Dermapter silvarum (Acari: Ixodidae). Exp. Appl. Acarol. 2021, 83, 271–283. [CrossRef]

45. Mulani, M.S.; Kabble, E.E.; Kumkar, S.N.; Taware, M.S.; Paradesi, K.R. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front. Microbiol. 2019, 10, 539. [CrossRef]

46. Wu, C.-L.; Hsueh, J.-Y.; Yip, B.-S.; Chih, Y.-H.; Peng, K.-L.; Cheng, J.-W. Antimicrobial Peptides Display Strong Synergy with Vancomycin against Vancomycin-Resistant E. faecium, S. aureus, and Wild-Type E. coli. Int. J. Mol. Sci. 2020, 21, 4578. [CrossRef]

47. Lakhundi, S.; Zhang, K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin. Microbiol. Rev. 2018, 31, e00020-18. [CrossRef] [PubMed]

48. Olto, M. Molecular insight into how MRSA is becoming increasingly dangerous. Virulence 2012, 3, 521–523. [CrossRef]

49. Zhou, M.; Jiang, W.; Xie, J.; Zhang, W.; Ji, Z.; Zou, J.; Cong, Z.; Xiao, X.; Gu, J.; Liu, R. Peptide-Mimicking Poly(2-oxazoline) Displaying Potent Antimicrobial Properties. ChemMedChem 2021, 16, 309–315. [CrossRef]

50. Munro-Moreno, M.; Suwalsky, M.; Patiño-González, E.; Fandiño-Devia, E.; Jemiola-Rzeminska, M.; Sztralka, K. Interaction of the antimicrobial peptide ∆M3 with the Staphylococcus aureus membrane and molecular models. Microbiol. Biophys. Acta BBA Biomembr. 2021, 1863, 183498. [CrossRef]
55. De Breij, A.; Riool, M.; Cordfunke, R.A.; Malanovic, N.; de Boer, L.; Koning, R.I.; Ravensbergen, E.; Franken, M.; van der Heijde, T.; Boekema, B.K.; et al. The antimicrobial peptide SAAP-148 combats drug-resistant bacteria and biofilms. Sci. Transl. Med. 2018, 10, 423. [CrossRef]

56. Fleeman, R.M.; Macias, L.A.; Brodbelt, J.S.; Davies, B.W. Defining principles that influence antimicrobial peptide activity against capsulated Klebsiella pneumoniae. Proc. Natl. Acad. Sci. USA 2020, 117, 27620–27626. [CrossRef]

57. Van der Weide, H.; Vermeulen-de Jongh, D.M.C.; van der Meijden, A.; Boers, S.A.; Kreft, D.; Ten Kate, M.T.; Falciani, C.; Pini, A.; Strandh, M.; Bakker-Woudenberg, I.; et al. Antimicrobial activity of two novel antimicrobial peptides AA139 and SET-M33 against clinically and genotypically diverse Klebsiella pneumoniae isolates with differing antibiotic resistance profiles. Int. J. Antimicrob. Agents 2019, 54, 159–166. [CrossRef]

58. Jaśkiewicz, M.; Neubauer, D.; Kazor, K.; Bartoszewska, S.; Kamysz, W. Antimicrobial Activity of Selected Antimicrobial Peptides Against Planktonic Culture and Biofilm of Acinetobacter baumannii. Probiotics Antimicrob. Proteins 2019, 11, 317–324. [CrossRef]

59. Liu, W.; Wu, Z.; Mao, C.; Guo, G.; Zeng, Z.; Fei, Y.; Wan, S.; Peng, J.; Wu, J. Antimicrobial Peptide Cee4 Eradicates the Bacteria of Clinical Carbapenem-Resistant Acinetobacter baumannii Biofilm. Front. Microbiol. 2020, 11, 1532. [CrossRef]

60. Neshani, A.; Sedighian, H.; Mirhosseini, S.A.; Ghazvini, K.; Zare, H.; Jahangiri, A. Antimicrobial peptides as a promising treatment option against Acinetobacter baumannii infections. Microb. Pathog. 2020, 146, 104238. [CrossRef] [PubMed]

61. Jung, C.-J.; Liao, Y.-D.; Hsu, C.-C.; Huang, T.-Y.; Chuang, Y.-C.; Chen, J.-W.; Kuo, Y.-M.; Kuo, Y.-M.; Chiu, J.-S. Identification of potential therapeutic antimicrobial peptide candidates against Acinetobacter baumannii in a mouse model of pneumonia. Sci. Rep. 2021, 11, 7318. [CrossRef] [PubMed]

62. Mwangi, J.; Yin, Y.; Wang, G.; Yang, M.; Li, Y.; Zhang, Z.; Lai, R. The antimicrobial peptide ZY4 combats multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii infection. Proc. Natl. Acad. Sci. USA 2019, 116, 26516–26522. [CrossRef] [PubMed]

63. Gorr, S.-U.; Brigm, H.; Anderson, J.; Hirsch, E. The antimicrobial peptide DGL13K is active against resistant gram-negative bacteria and subinhibitory concentrations stimulate bacterial growth without causing resistance. bioRxiv 2020. [CrossRef]

64. Hirt, H.; Gorr, S.-U. Antimicrobial peptide GL13K is effective in reducing biofilms of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 2013, 57, 4903–4910. [CrossRef]

65. Ghosh, C.; Harmouche, N.; Bechinger, B.; Haldar, J. Aryl-Alkyl-Lysines Interact with Anionic Lipid Components of Bacterial Cell Envelope Eliciting Anti-Inflammatory and Antibiofilm Properties. ACS Omega 2018, 3, 9182–9190. [CrossRef] [PubMed]

66. Santajit, S.; Indrawattana, N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. BioMed Res. Int. 2016, 2016, 2475067. [CrossRef]

67. Bechinger, B.; Juhl, D.W.; Glanttard, E.; Aisenbrey, C. Revealing the Mechanisms of Synergistic Action of Two Magainin Antimicrobial Peptides. Front. Med Technol. 2020, 2, hal-03108924f. [CrossRef]

68. Desbois, A.P.; Gemmell, C.G.; Coote, P.J. In vivo efficacy of the antimicrobial peptide ranalexin in combination with the endopeptidase lycsostaphin against wound and systemic meticillin-resistant Staphylococcus aureus (MRSA) infections. Int. J. Antimicrob. Agents 2010, 35, 559–565. [CrossRef]

69. Jangra, M.; Raka, V.; Nandanwar, H. In Vitro Evaluation of Antimicrobial Peptide Tridecaptin M in Combination with Other Antibiotics against Multidrug Resistant Acinetobacter baumannii. Molecules 2020, 25, 3255. [CrossRef]

70. Vanhoye, D.; Bruston, F.; Nicolas, P.; Amiche, M. Antimicrobial peptides from hylid and ranin frogs originated from a 150-million-year-old ancestral precursor with a conserved signal peptide but a hypermutable antimicrobial domain. Proc. Natl. Acad. Sci. USA 2020, 117, 1646–1655. [CrossRef] [PubMed]

71. Zaïri, A.; Ferrièrè, L.; Latour-Lambert, P.; Beloin, C.; Tangy, F.; Ghigo, J.M.; Hani, K. In vitro activities of dermaseptins K4S4 and K4K20S4 against Escherichia coli, and Pseudomonas aeruginosa planktonic growth and biofilm formation. Antimicrob. Agents Chemother. 2014, 58, 2221–2228. [CrossRef]

72. Yin, X.; Heeney, D.; Risengya, F.; Golomb, B.; Griffey, S.; Marco, M. Bacteriocin biosynthesis contributes to the anti-inflammatory capacities of probiotic Lactobacillus plantarum. Benef. Microbes 2019, 8, 333–344. [CrossRef]

73. Wu, M.; Hancock, R.E. Interaction of the cyclic antimicrobial cationic peptide bacterenecin with the outer and cytoplasmic membrane. J. Biol. Chem. 1999, 274, 29–35. [CrossRef] [PubMed]

74. Oo, T.Z.; Cole, N.; Garthwaite, L.; Willcox, M.D.; Zhu, H. Evaluation of synergistic activity of bovine lactoferrin with antibiotics in corneal infection. J. Antimicrob. Chemother. 2010, 65, 1243–1251. [CrossRef] [PubMed]

75. Field, D.; Seiling, N.; Cotter, P.D.; Ross, R.P.; Hill, C. Synergistic Nisin-Polymyxin Combinations for the Control of Pseudomonas Biofilm Formation. Front. Microbiol. 2016, 7, 1713. [CrossRef] [PubMed]

76. Jahangiri, A.; Neshani, A.; Mirhosseini, S.A.; Ghazvini, K.; Zare, H.; Sedighian, H. Synergistic effect of two antimicrobial peptides, Nisin and P10 with conventional antibiotics against extensively drug-resistant Acinetobacter baumannii and colistin-resistant Pseudomonas aeruginosa isolates. Microb. Pathog. 2021, 150, 104700. [CrossRef] [PubMed]

77. Portelinha, J.; Angeles-Boza, A.M. The Antimicrobial Peptide Gad-1 Clears Pseudomonas aeruginosa Biofilms under Cystic Fibrosis Conditions. Chembiochem Eur. J. Chem. Biol. 2021, 22, 1646–1655. [CrossRef] [PubMed]

78. Clarke, E.A. What is Preventive Medicine? Can. Fam Physician 1974, 20, 65–68. [PubMed]

79. Giacometti, A.; Cirioni, O.; Ghiselli, R.; Goffi, L.; Mocchegiani, F.; Riva, A.; Scalise, G.; Saba, V. Polycationic peptides as prophylactic agents against meticillin-susceptible or meticillin-resistant Staphylococcus epidermidis vascular graft infection. Antimicrob. Agents Chemother. 2000, 44, 3306–3309. [CrossRef] [PubMed]
80. Mateescu, M.; Baixe, S.; Garmier, T.; Jierry, L.; Ball, V.; Haikel, Y.; Metz-Boutigue, M.H.; Nardin, M.; Schaaf, P.; Etienne, O.; et al. Antibi granted Peptide-Based Gel for Prevention of Medical Implanted-Device Infection. PLoS ONE 2015, 10, e0145143. [CrossRef]

81. Monteiro, C.; Costa, F.; Piritülü, A.M.; Tejesvi, M.V.; Martins, M.C.L. Prevention of urinary catheter-associated infections by coating antimicrobial peptides from crownberry endophytes. Sci. Rep. 2019, 9, 10753. [CrossRef]

82. Niu, J.Y.; Yin, I.X.; Wu, W.K.K.; Li, Q.-L.; Mei, M.L.; Chu, C.H. Antimicrobial peptides for the prevention and treatment of dental caries: A concise review. Arch. Oral Biol. 2021, 122, 105022. [CrossRef]

83. Volejníková, A.; Melichertík, P.; Nešuta, O.; Vanková, E.; Bednárová, L.; Rybáček, J.; Čeřovský, V. Antimicrobial peptides prevent bacterial biofilm formation on the surface of polymethylmethacrylate bone cement. J. Med. Microbiol. 2019, 68, 961–972. [CrossRef]

84. Vestby, L.K.; Gronsseth, T.; Simm, R.; Nesse, L.L. Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics 2020, 9, 59. [CrossRef] [PubMed]

85. Donlan, R.M. Biofilms: Microbial life on surfaces. Emerg. Infect. Dis. 2002, 8, 881–890. [CrossRef]

86. Muhammad, M.H.; Idris, A.L.; Fan, X.; Guo, Y.; Yu, Y.; Jin, X.; Qu, J.; Guan, X.; Huang, T. Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. Front. Microbiol. 2020, 11, 928. [CrossRef] [PubMed]

87. Rabin, N.; Zheng, Y.; Opoku-Temeng, C.; Du, Y.; Bonsu, E.; Sintim, H.O. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med. Chem. 2015, 7, 493–512. [CrossRef] [PubMed]

88. Bogino, P.C.; Olivera, M.d.l.M.; Sorroche, F.G.; Giordano, W. The role of bacterial biofilms and surface components in plant-bacterial associations. Int. J. Mol. Sci. 2013, 14, 15838–15859. [CrossRef] [PubMed]

89. Galderio, E.; Lombardi, L.; Falanga, A.; Libralato, G.; Guida, M.; Carotenuto, R. Biofilms: Novel Strategies Based on Antimicrobial Peptides. Pharmaceuticals 2019, 11, 322. [CrossRef] [PubMed]

90. Percival, S.L.; Suleman, L.; Vuotto, C.; Donelli, G. Healthcare-associated infections, medical devices and biofilms: Risk, tolerance and control. J. Med. Microbiol. 2015, 64, 323–334. [CrossRef] [PubMed]

91. Jang, C.H.; Park, H.; Cho, Y.B.; Choi, C.H. Effect of vancomycin-coated tympanostomy tubes on methicillin-resistant Staphylococcus aureus biofilm formation: In vitro study. J. Laryngol. Otol. 2010, 124, 594–598. [CrossRef]

92. Wongkaewkhiaw, S.; Taweechaisupapong, S.; Thanavitrananich, S.; Bolscher, J.G.M.; Nazmi, K.; Anutrakunchai, C.; Chareonsudjai, S.; Kanthawong, S. D-LL-31 enhances biofilm-eradicating effect of currently used antibiotics for chronic rhinosinusitis and its immunomodulatory activity on human lung epithelial cells. PLoS ONE 2020, 15, e0243315. [CrossRef]

93. Wongkaewkhiaw, S.; Taweechaisupapong, S.; Anutrakunchai, C.; Nazmi, K.; Bolscher, J.G.M.; Wongratanacheewin, S.; Kanthawong, S. D-LL-31 in combination with ceftazidime synergistically enhances bactericidal activity and biofilm destruction in Burkholderia pseudomallei. Biofouling 2019, 35, 573–584. [CrossRef]

94. Guo, Z.; Wang, Y.; Tan, T.; Ji, Y.; Hu, J.; Zhang, Y. Antimicrobial d-Peptide Hydrogels. ACS Biomater. Sci. Eng. 2021, 7, 1703–1712. [CrossRef]

95. Li, M.; Yu, D.H.; Cai, H. The synthetic antimicrobial peptide KLKL5KLK enhances the protection and efficacy of the combined DNA vaccine against Mycobacterium tuberculosis. DNA Cell Biol. 2008, 27, 405–413. [CrossRef] [PubMed]

96. Olafsdottir, T.A.; Lingnau, K.; Nagy, E.; Jonsdottir, I. IC31, a two-component novel adjuvant mixed with a conjugate vaccine for prophylactic DNA vaccine against Mycobacterium tuberculosis. DNA Cell Biol. 2009, 28, 105022. [CrossRef]

97. Micoli, F.; Bagnoli, F.; Rappuoli, R.; Serruto, D. The role of vaccines in combatting antimicrobial resistance. Acc. Chem. Res. 2013, 46, 1583–1593. [CrossRef] [PubMed]

98. Mahendran, A.S.K.; Lim, Y.S.; Fang, C.-M.; Loh, H.-S.; Le, C.F. The Potential of Antiviral Peptides as COVID-19 Therapeutics. ACS Pharmcol. Transl. Sci. 2020, 3, 780–782. [CrossRef] [PubMed]

99. Pardoux, É.; Boturyń, D. Antimicrobial Peptides as Probes in Biosensors Detecting Whole Bacteria: A Review. Molecules 2020, 25, 1998. [CrossRef]

100. Bakovic, A.; Risner, K.; Bhalla, N.; Alem, F.; Chang, T.L.; Weston, W.; Harness, J.A.; Narayanan, A. Brilacidin, a COVID-19 Drug Candidate, Exhibits Potent In Vitro Antiviral Activity Against SARS-CoV-2. bioRxiv 2020. [CrossRef] [PubMed]

101. Bakovic, A.; Risner, K.; Bhalla, N.; Alem, F.; Chang, T.L.; Weston, W.; Harness, J.A.; Narayanan, A. Brilacidin, a COVID-19 Drug Candidate, Exhibits Potent In Vitro Antiviral Activity Against SARS-CoV-2. bioRxiv 2020. [CrossRef] [PubMed]

102. Database of Antimicrobial Activity and Structure of Peptides. Available online: https://dbaasp.org/ (accessed on 21 June 2021).
109. Greco, L.; Molchanova, N.; Holmedal, E.; Jenssen, H.; Hummel, B.D.; Watts, J.L.; Håkansson, J.; Hansen, P.R.; Svenson, J. Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. *Sci. Rep.* 2020, 10, 13206. [CrossRef] [PubMed]

110. Moncla, B.J.; Pryke, K.; Rohan, L.C.; Graebling, P.W. Degradation of naturally occurring and engineered antimicrobial peptides by proteases. *Adv. Biosci. Biotechnol.* 2011, 2, 404–408. [CrossRef] [PubMed]

111. Böttger, R.; Hoffmann, R.; Knappe, D. Differential stability of therapeutic peptides with different proteolytic cleavage sites in blood, plasma and serum. *PLoS ONE* 2012, 7, e01178943. [CrossRef]

112. Puarini, D.; Santasabuj, D. Mammalian Antimicrobial Peptides: Promising Therapeutic Targets Against Infection and Chronic Inflammation. *Curr. Top. Med. Chem.* 2016, 16, 99–129. [CrossRef]

113. Pfalzgraff, A.; Brandenburg, K.; Weindl, G. Antimicrobial Peptides and Their Therapeutic Potential for Bacterial Skin Infections and Wounds. *Front. Pharmacol.* 2018, 9, 281. [CrossRef]

114. Atefyekta, S.; Blomstrand, E.; Rajasekharan, A.K.; Svensson, S.; Trobos, M.; Hong, J.; Webster, T.J.; Thomsen, P.; Andersson, M. Antimicrobial Peptide-Functionalized Mesoporous Hydrogels. *ACS Biomater. Sci. Eng.* 2021, 7, 1693–1702. [CrossRef] [PubMed]

115. Boto, A.; Pérez de la Lastra, J.M.; González, C.C. The Road from Host-Defense Peptides to a New Generation of Antimicrobial Drugs. *Molecules* 2018, 23, 311. [CrossRef]

116. Luong, H.X.; Thanh, T.T.; Tran, T.H. Antimicrobial peptides—Advances in development of therapeutic applications. *Life Sci.* 2020, 260, 118407. [CrossRef]

117. Nuding, S.; Gersemann, M.; Hosaka, Y.; Konietzny, S.; Schaefer, C.; Beisner, J.; Schroeder, B.O.; Ostaff, M.J.; Saigenji, K.; Ott, G.; et al. Gastric antimicrobial peptides fail to eradicate Helicobacter pylori infection due to selective induction and resistance. *PLoS ONE* 2013, 8, e738367. [CrossRef]

118. Joo, H.-S.; Fu, C.-I.; Otto, M. Bacterial strategies of resistance to antimicrobial peptides. Philosophical transactions of the Royal Society of London. *Ser. B Biol. Sci.* 2016, 371, 20150292. [CrossRef]

119. Min, C.; Ohta, K.; Kajiya, M.; Zhu, T.; Sharma, K.; Shin, J.; Mawardi, H.; Howait, M.; Hirschfeld, J.; Bahammam, L.; et al. The antimicrobial activity of the appetite peptide hormone ghrelin. *Peptides* 2012, 36, 151–156. [CrossRef] [PubMed]

120. Koo, H.B.; Seo, J. Antimicrobial peptides under clinical investigation. *PLoS ONE* 2012, 7, e34122. [CrossRef]

121. Obuobi, S.; Tay, H.K.-L.; Tran, N.D.T.; Selvarajan, V.; Khara, J.S.; Wang, Y.; Ee, P.L.R. Facile and efficient encapsulation of antimicrobial peptides via crosslinked DNA nanostructures and their application in wound therapy. *J. Control. Release* 2019, 313, 120–130. [CrossRef]

122. Molhoek, E.M.; van Dijk, A.; Veldhuizen, E.J.; Haagsman, H.P.; Bikker, F.J. Improved proteolytic stability of chicken cathelicidin-2 derived peptides by D-amino acid substitutions and cyclization. *Peptides* 2011, 32, 875–880. [CrossRef] [PubMed]

123. Moorcroft, S.C.T.; Roach, L.; Jayne, D.G.; Ong, Z.Y.; Evans, S.D. Nanoparticle-Loaded Hydrogel for the Light-Activated Release and Photothermal Enhancement of Antimicrobial Peptides. *ACS Appl. Mater. Interfaces* 2020, 12, 24544–24554. [CrossRef]

124. Sun, C.; Gu, L.; Hussain, M.A.; Chen, L.; Lin, L.; Wang, H.; Pang, S.; Jiang, C.; Jiang, Z.; Hou, J. Characterization of the Bioactivity and Mechanism of Bactericidal Derivatives Against Food-Pathogens. *Front. Microbiol.* 2019, 10, 2593. [CrossRef]

125. Veltri, D.; Kamath, U.; Shehu, A. Improving Recognition of Antimicrobial Peptides and Target Selectivity through Machine Learning and Genetic Programming. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 2017, 14, 300–313. [CrossRef]

126. Yurkova, M.S.; Zenin, V.A.; Sadykhov, E.G.; Fedorov, A.N. Dimerization of Antimicrobial Peptide Polyphemus I into One Polypeptide Chain: Theoretical and Practical Consequences. *Appl. Biochem. Microbiol.* 2020, 56, 893–897. [CrossRef]

127. Irazazabal, L.N.; Porto, W.F.; Ribeiro, S.M.; Casale, S.; Humblot, V.; Ladram, A.; Franco, O.L. Selective amino acid substitution reduces cytotoxicity of the antimicrobial peptide mastoparan. *Biochim. Biophys. Acta* 2016, 1859, 2699–2708. [CrossRef] [PubMed]

128. Drayton, M.; Kizhakkedathu, J.N.; Straus, S.K. Towards Robust Delivery of Antimicrobial Peptides to Combat Bacterial Resistance. *Molecules* 2020, 25, 3048. [CrossRef]

129. Sarma, P.; Mahendiratta, S.; Prakash, A.; Medihi, B. Specifically targeted antimicrobial peptides: A new and promising avenue in selective antimicrobial therapy. *Indian J. Pharm.* 2018, 50, 1–3. [CrossRef]

130. Guo, L.; Edlund, A. Targeted Antimicrobial Peptides: A Novel Technology to Eradicate Harmful Streptococcus Mutans. *J. Calif. Dent. Assoc.* 2017, 45, 557–564.

131. Peng, J.; Qiu, S.; Jia, F.; Zhang, L.; He, Y.; Zhang, E.; Sun, M.; Deng, Y.; Guo, Y.; Xu, Z.; et al. The introduction of L-phenylalanine into antimicrobial peptide protonecin enhances the selective antibacterial activity of its derivative phe-Prt against Gram-positive bacteria. *Amino Acids* 2021, 53, 23–32. [CrossRef]

132. Liu, Y.; Shen, T.; Chen, L.; Zhou, J.; Wang, C. Analogs of the Cathelicidin-Derived Antimicrobial Peptide PMAP-23 Exhibit Improved Stability and Antibacterial Activity. *Probiotika Antimicrob. Proteins* 2021, 13, 273–286. [CrossRef]

133. Chen, X.; Zhang, L.; Wu, Y.; Wang, L.; Ma, C.; Xi, X.; Bininda-Emonds, O.R.P.; Shaw, C.; Chen, T.; Zhou, M. Evaluation of the bioactivity of a mastoparan peptide from wasp venom and of its analogues designed through targeted engineering. *Int. J. Biol. Sci.* 2018, 14, 599–607. [CrossRef]