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Antimicrobial Peptides and Peptidomimetics – Potent Therapeutic Allies for Staphylococcal Infections

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Abstract

The pervasiveness of bacterial resistance to conventional antibiotics, particularly those associated with staphylococcal infections, has become a global epidemic. However, research involving antimicrobial peptides (AMPs) and their synthetic analogues has unearthed a potentially novel class of antibacterials for the treatment of an array of diseases caused by pathogenic bacteria, including staphylococci. AMPs have several unique advantages over traditional antibiotics including the projected slow emergence of bacterial resistance to these agents and their capability to modulate the host immune response to infection. Unfortunately, their susceptibility to proteolytic degradation, loss of antimicrobial activity due to serum binding or physiological concentration of salts, and toxicity to host tissues has limited their use as systemic agents thus far. Additionally, the presence of economic and regulatory obstacles have hindered the translation of AMPs, as antimicrobials, from the bench to the clinic. The present review delves further into the benefits and challenges of utilizing AMPs as antibacterial agents (particularly for staphylococcal infections), methods which have been utilized to overcome their limitations, their successes and failures in clinical trials, and future avenues for researchers to pursue to develop AMPs as novel therapeutic allies in the treatment of bacterial infections.

Keywords

Antimicrobial peptides; peptidomimetics; antibiotics; multidrug-resistance; staphylococci; MRSA; bacterial resistance; antibacterial; immunomodulatory agents

1. INTRODUCTION

Multidrug-resistant pathogens pose a significant global public health challenge and have been identified in every geographic region of the world (1). Annually, in the United States alone, these pathogens negatively impact the lives of over two million people at a cost of $20 billion to the healthcare system and result in over 23,000 deaths (2). Of these fatalities, nearly half are attributed to a single bacterial pathogen, methicillin-resistant *Staphylococcus aureus*. While once restricted to the healthcare setting (referred to as healthcare-associated MRSA or HA-MRSA), MRSA infections have become a major problem in the community

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(referred to as community-acquired MRSA or CA-MRSA) affecting a diverse population including healthcare workers, prison inmates, members of the military, athletes, the homeless population, intravenous drug users, newborn babies, and young children (3–12). Furthermore, CA-MRSA infections are typically associated with more severe morbidity and mortality than their HA-MRSA counterparts (13). While CA-MRSA is a leading cause of skin and soft-tissue infections, MRSA has also been associated with more complicated medical diseases including necrotizing pneumonia, osteomyelitis, and sepsis (14–18).

For nearly 70 years, natural product antibiotics and their synthetic analogues have been the gold standard for treatment of infections caused by bacterial pathogens. However, the emergence of bacterial strains exhibiting resistance to numerous antibiotics has resulted in treatment failure. Indeed, clinical isolates of both CA-MRSA and HA-MRSA have been documented which exhibit resistance to nearly all antibiotic classes including the β-lactams, macrolides, quinolones, tetracyclines, and lincosamides (19–23). Further exacerbating the problem, are strains have emerged that exhibit resistance to both first-line antibiotics and drugs deemed agents of last resort (such as linezolid and vancomycin) (24–26). Though prudent use of effective antimicrobials is a critical step to alleviate complications and costs associated with MRSA infections, alternative strategies to combat this global challenge must be explored. One potential alternative for novel therapeutic agents to treat infections caused by multidrug-resistant pathogens that has shown significant promise in recent years is the isolation of natural and development of synthetic antimicrobial peptides (AMPs).

Since their initial discovery, AMPs have been isolated from bacteria (termed bacteriocins), marine animals, plants, birds, insects, frogs, and the human innate immune response (27–30). AMPs traditionally consist of a short chain of 12 to 100 amino acids linked by peptide bonds (31). They typically are cationic (with at least two positive charges) which permits their interaction with negatively-charged components present in the bacterial membrane and cell wall; furthermore, AMPs are amphipathic which is thought to permit their ability to target and partition into bacterial cell membranes (30, 32–34). In addition to their membrane-disrupting abilities, antibacterial peptides have also been shown to attack intracellular targets in bacteria including inhibiting macromolecular synthesis, inhibiting nucleic acid or protein synthesis, binding directly to nucleic acids, or interfering with cell wall synthesis (31, 35–37).

In addition to possessing potent antibacterial activity, AMPs have several unique advantages over traditional antibiotics which led to the excitement in trying to isolate and develop synthetic analogues of peptides as novel therapeutic agents. Amongst these selective advantages include a low rate of bacterial resistance emerging, ability to neutralize virulence factors released by pathogens which can trigger a pro-inflammatory immune response in the host (leading to sepsis or toxic shock syndrome), and the proven ability of AMPs (or more specifically, host defense peptides) to stimulate or modulate the host immune response which consequently helps infected hosts to achieve more rapid healing and repair of compromised tissues (38). However while they possess several advantages over traditional antibiotics, AMPs also have several critical limitations that have hindered their ability to be translated into clinical use, particularly for treatment of invasive infections. Among the weaknesses identified thus far for AMPs as antimicrobials include toxicity to host tissues,

rapid degradation by proteases, sensitivity to changes in salt concentration, and extensive binding to serum proteins (29, 31, 39–42). Though much research has been undertaken to overcome these limitations, a critical analysis of these findings is needed to transform these promising novel antibacterials into therapeutic compounds capable of being used in the clinic, particularly for infections attributed to multidrug-resistant staphylococci.

An extensive review of published literature revealed more than twenty AMPs that possess activity against staphylococci (both *in vitro* and *in vivo* in an animal study) have been isolated/developed in the past two decades. The source (from where each peptide was derived), amino acid sequence, *in vitro* biological activity (denoted as the minimum inhibitory concentration (MIC) capable of inhibiting bacterial growth), and toxicity (to host tissues) of the most promising peptides are outlined in Table 1. The present review will delve into the potential use of AMPs as novel anti-staphylococcal agents by focusing on their unique modes of action against bacteria, advantages and limitations of AMPs as therapeutic agents, current AMPs in clinical trials for the treatment of staphylococcal infections, and future avenues, clinically, for the development of AMPs as anti-staphylococcal agents.

2. MECHANISM OF ACTIONS OF AMPs

Attaining a proper understanding of the biological target upon which antibacterials exhibit their activity is critical to propel them into the latter stages of clinical development. With regards to AMPs, the primary mechanism of action involves targeted disruption of the bacterial cytoplasmic membrane (102). The positive charge present in certain residues within the AMP serves as a point of attraction towards anionic components present on the bacterial cell membrane (such as the phospholipid head-groups). This electrostatic interaction ultimately leads to perturbation of the bacterial cell membrane (102). Membrane disruption often occurs rapidly and is very non-specific. Four predominant models (the toroidal-pore model, the barrel-stave model, the aggregate model, and the carpet model) have been proposed to describe the events that occur as a result of interaction of AMPs with the bacterial membrane (103–105). These models have been classified based upon the method the AMP employs to disrupt the membrane which can occur via (i) transient channel formation, (ii) disintegration of the integrity of the bacterial membrane, or (iii) transport through the membrane (102). Irrespective of the method utilized, eventually AMPs cause membrane depolarization and bacterial lysis. The presence of negatively-charged phospholipids in the bacterial membrane is an important factor for the selective action of AMPs upon bacteria over eukaryotic cells, where neutrally charged lipids are found to be predominant in mammalian cells (31).

Antimicrobial peptides are also capable of targeting important components that play a role in bacterial cell wall synthesis. Nisin, a bacteriocin isolated from *Lactococcus lactis*, binds to lipid II and prevents peptidoglycan formation, resulting in inhibition of cell wall synthesis (106); additionally, nisin also causes membrane disruption. Interestingly, vancomycin also targets the same biosynthetic pathway for cell wall synthesis as nisin; however, this peptide is thought to interact with a different moiety within lipid II, as compared to vancomycin. This is further supported by the fact that vancomycin-resistant strains of *S. aureus* (VRSA) are susceptible to nisin (81). Plectasin, a fungal defensin, also binds to lipid II and

inhibits bacterial cell wall biosynthesis (107). Therefore, in addition to causing membrane depolarization, it is likely that AMPs attack more than one biological target in bacteria. This complexity in their mechanism of action makes it very difficult to isolate bacterial mutants exhibiting resistance to peptides, as will be explained further in the latter portions of this review (108).

In addition to membrane disruption and interfering with bacterial cell wall synthesis, well-established studies have identified that peptides are also capable of attacking internal targets present in the bacterial cytoplasm. These particular peptides, at the minimal effective concentration, do not permeabilize the bacterial membrane but instead, they translocate into the cells and moderate cell death by targeting critical processes inside the bacterial cell. For example, dermaseptin, an AMP isolated from frog skin, inhibits RNA and DNA synthesis without causing membrane depolarization (31). This mechanism of macromolecular synthesis disruption appears similar to that of the antibiotic rifampicin, a RNA polymerase inhibitor (109). Similarly, PR-39, an antimicrobial peptide identified from the pig intestine, and pleurocidin, isolated from fish, inhibit DNA replication and protein synthesis (35, 109). Human Neutrophil Peptide-1 (HNP-1) has also been shown to inhibit nucleic acid synthesis and protein synthesis (110). Indolicidin, derived from bovine neutrophils, blocks DNA synthesis in addition to causing cell membrane disruption (111, 112).

3. IMMUNOMODULATORY ACTIVITY OF AMPs

3.1. Cytokine regulation and immune cell chemotaxis

As described above, AMPs possess several unique methods to exhibit their antibacterial action on bacteria ultimately resulting in the resolution of an infection. However, an alternative mechanism by which AMPs play a role in the clearance of a bacterial infection involves a direct modulation of the host immune response against an invading pathogen. Human defensin peptides form an important component for the immune response against infections, which is supported by the fact that mice lacking the AMP cathelicidin are found to be more susceptible to bacterial infection (113). The ability to modulate host immune responses, either by inhibition or stimulation, varies significantly based on numerous factors; however, this has proven to be a very useful strategy for consideration for the prevention and treatment of bacterial infections. For example, innate defense regulator peptide-1 (IDR-1) has no innate antimicrobial activity *in vitro*, but it has been shown to protect mice from antibiotic-resistant *S. aureus* infection (114). IDR-1 selectively suppresses pro-inflammatory cytokines, such as TNF-α and IL-6 at the site of infection, and IDR-1 also increases the production of the anti-inflammatory cytokine IL-10 in the blood (114). Additionally, IDR-1 stimulates production of different chemokines such as RANTES and MCP-1 which recruit both monocytes and macrophages to the site of infection (114). RANTES and MCP-1 play an important role in protection against infection (114–116). IDR-1 also protects monocytes from the cytotoxic action of *S. aureus* (114). Given the excellent immunomodulatory properties of IDR-1, this agent has strong potential for use as a therapeutic agent to clear *S. aureus* infections. In addition to IDR-1, the peptide hLF1–11, a lactoferrin derivative, exhibits immunomodulatory activity in its ability to bind to human

monocytes and subsequently inhibit generation of the myeloperoxidase (MPO) enzyme, a negative regulator of IL-10 (117). hLF1–11 also enhances granulocyte-macrophage colony-stimulating factor (GM-CSF)-derived monocyte differentiation to macrophages which exhibit increased antimicrobial activity against *S. aureus* (118).

Cathelicidins derived from humans (LL-37), cows (indolicidin), and pigs (PR-39) have been shown to facilitate the recruitment of various immune cells such as neutrophils, monocytes, T cells, and mast cells to the site of infection (119–121). LL-37 induces various chemokines such as MCP-1, RANTES, and IL-8 to promote chemotaxis of immune cells (122–124). Cathelicidins from humans and cows also have been shown to be capable of suppressing pro-inflammatory cytokine production (including cytokines such as IL-1β and TNF-α) while also increasing anti-inflammatory cytokine production (including IL-10) (119, 120, 125). IB-367 from porcine neutrophils increases the migration of CD11b and Gr-1 positive cells to the site of infection and enhances bacterial clearance (59). Thus, the overall net result of the immunomodulatory functions of peptides and AMPs leads to the control of inflammatory responses in the affected host without altering or compromising the immune responses that are essential for the clearance of the bacterial infection. This dual role of AMPs as antibacterial and immunomodulatory agents has led to their exploration as alternative therapeutics for the treatment of multi-drug resistant *S. aureus* infections.

3.2. Wound healing and angiogenesis

The utilization of antimicrobial peptides as topical agents to treat both acute and chronic staphylococcal infections (including LTX-109 and Brilacidin (PMX-30063)) has been steadily gaining momentum as can be seen by the increasing number of AMPs that are entering into clinical trials. AMPs can play a direct role in wound healing and angiogenesis, two desirable physiological effects for the resolution of staphylococcal skin infections. A classic example of this are the human defensin peptides (hBDP1–4) which have been shown to enhance chemotaxis of immune cells to wounded tissues and increase keratinocyte migration and proliferation, thereby stimulating tissue regeneration (126, 127). This is further supported by the fact that the lack of hBDs leads to delayed tissue regeneration and impaired re-epithelialization in chronic wounds (128, 129).

Chronic wound healing in diabetic foot ulcer patients is a challenging medical issue, particularly wounds infected with bacteria. *S. aureus*-infected diabetic foot ulcers are common and hard to treat; proper healing of such infected wounds is often complicated by impaired functioning of immune cells (130–136). As stated earlier, hBDPs are known to accelerate healing of wounds including those present in patients with diabetic foot ulcers. For example, one study demonstrated that human β-defensin 3 (hBD3) enhances tissue regeneration and closure of an infected wound in a diabetic porcine model (137). In addition to this, considerably higher hBD3 expression was found in human primary keratinocytes treated with heat inactivated *S. aureus*, which supports the fact that hBD3 may aid in healing of infected skin wounds (93). Hence, considering their anti-inflammatory, wound healing, and angiogeneic properties, AMPs may have potential for further exploration for the treatment of *S. aureus* infected chronic wounds.

4. ADVANTAGES OF USING PEPTIDES FOR THE TREATMENT OF BACTERIAL INFECTIONS

In addition to their capability to modulate the host immune response, antimicrobial peptides possess several unique advantages over empirical antibiotics used for the treatment of multidrug-resistant staphylococcal infections. These advantages include (1) potent bactericidal activity and (2) low frequency of bacterial resistance emerging. Combined with AMPs' anti-inflammatory properties, these unique assets permit AMPs to be utilized in multiple unique therapeutic applications as will be discussed in more detail below and in latter portions of this review.

4.1. Potent antibacterial and rapid bactericidal activity against Gram-positive pathogens, including *S. aureus*

Numerous peptides have shown potent *in vitro* antimicrobial activity against important Gram-positive pathogens, including MRSA. Indeed, many published reports have indicated that these AMPs have activity that is similar to or better than conventional antibiotics including vancomycin (28). Nisin has been used as a food preservative for more than 50 years with limited development of bacterial resistance to this agent (31). This same AMP has been shown to inhibit the growth of Gram-positive bacteria at concentrations in the nanomolar range (31). In addition to nisin, AMPs derived from the horseshoe crab (including polyphemusin) have been found to exhibit excellent antibacterial activity, inhibiting bacterial growth at concentrations below 2 μg/mL (138). Maganins, isolated from the skin of frogs and other amphibians, have also shown powerful antimicrobial activity against Gram-positive bacteria. They were the template for the development of the first clinical AMPs but were ultimately found to be unsuccessful for use as therapeutic agents (31). Cathelicidins, isolated from the immune system of many mammals (such as BMAP-28, a bovine AMP), have also been shown to possess excellent antibacterial activity against Gram-positive pathogens (139).

When peptides were examined for their rate of bactericidal activity, many were found to be capable of complete elimination of an initial bacterial inoculum within a few hours. Pexiganan (a synthetic derivative of magainin currently in clinical trials), at a concentration of 256 μg/mL, was shown in one study to completely eliminate *S. aureus* ATCC 29213 (starting inoculum of 10^6 CFU/mL) within two hours (140). Importantly, this peptide retained its bactericidal activity against isolates of *S. aureus* exhibiting resistance to ofloxacin, oxacillin, cefazolin, and imipenem, an additional benefit that has been noted with AMPs. Furthermore the porcine-derived peptide, protegrin-1 (PG-1), at concentrations equivalent to 1 × or 2.5 × MIC, was found to be capable of producing a 3 to 5-$\log_{10}$ reduction in MRSA (both in stationary and log phases of growth) growth, within 8 minutes of exposure, via a standard time-kill assay (63).

4.2. Low frequency of bacterial resistance emerging

As noted by Maria Papagianni in her thorough review of ribosomally-synthesized AMPs, peptide-based antibiotics are thought to be a potential solution to the burgeoning issue of bacterial resistance that has emerged to traditional antibiotics (34). Bacterial resistance to

AMPs is unlikely to occur rapidly (as compared to conventional antibiotics), in part, due to the mode of action of these peptides. Given that peptides act on critical structural features present on the surface of bacterial cells (namely the cell membrane), in addition to attacking multiple intracellular targets, this complex multi-target attack on bacteria supports the notion that resistance to AMPs will be slow to develop (31). This postulate has been supported by recent publications examining the propensity of microbial resistance to emerge to different AMPs after serial passage of these compounds at subinhibitory concentrations, as discussed below.

Ge *et al* noted that when an isolate of *S. aureus* was exposed to a subinhibitory concentration of pexiganan over eight passages, only a slight increase (from 5.8 μg/mL to 8.3 μg/mL) in the MIC was found (140). Interestingly, no increase in the MIC was observed when the same experiment was repeated for coagulase-negative staphylococci, confirming that bacterial resistance to this particular peptide is slow to develop. A second published report by Steinberg *et al* discovered that when the peptide PG-1 (at ½ × MIC) was repeatedly exposed to the same strain of MRSA (for 18 passages), no resistance was observed (63). In contrast, when the same analysis was completed with the antibiotic norfloxacin, an 85-fold increase in MIC was observed. Moreover, cross-resistance between peptides and antibiotics (or peptides with other peptides) even after exposure of bacteria to multiple passages of AMPs has not been observed in multiple studies (108, 141). This is in contrast to traditional antibiotics where cross-resistance between multiple antibiotics has been observed in a single drug-resistant bacterial strain; a classic example of this is bacterial production of extended spectrum β-lactamases which confers cross-resistance to multiple antibiotic classes including penicillins and cephalosporins (142, 143). It is also worthy to note here that the peptide nisin, as mentioned earlier, has been used commercially for many years as a food preservative with limited reports of bacterial resistance to this agent being published. These results collectively support the proposition that bacterial resistance, in particular by MRSA, to AMPs would be expected to emerge much slower than what has been observed with traditional antibiotics. Additionally, should resistance to a particular AMP be noted, simple modifications in the structural residues can be utilized to overcome this issue, rendering the AMP still effective. Furthermore, should resistance develop to the antibacterial component of AMPs (via structural modifications to the biological cell membrane, activation of efflux pumps, or through bacterial proteolytic degradation of AMPs), it doesn't affect their potential to be used alternatively as immunomodulatory agents (to stimulate the host immune response to eliminate these pathogens).

4.3. Anti-inflammatory activity of AMPs

In addition to the potent antibacterial activities reported for many natural and synthetic peptides, several peptides have a dual advantage as antibacterial and anti-inflammatory agents. Of these published reports, Zhang *et al* demonstrated that synthetic peptides (such as HB43, HB55, and HBPM4) screened for activity against pathogens (including *S. aureus*) which commonly infect cystic fibrosis patients, possessed excellent antibacterial activity against MRSA (MIC of the three best peptides was 4 μg/mL) (108). Inflammation is a major problem in patients afflicted with cystic fibrosis and is thought to contribute significantly to lung tissue destruction. Interestingly, the authors also discovered that one

of their peptides (HBCM2) produced a significant reduction in inflammation *in vivo* in a murine ear edema model. Furthermore, another study found that the peptide HBPM4 was capable of neutralizing endotoxin (108), which can lead to severe clinical diseases including sepsis or septic shock. One drawback of conventional antibiotics is they can actually stimulate the release of endotoxins by bacteria (144, 145); thus peptides possessing both anti-inflammatory activity and the capability to inhibit toxin production possess a unique advantage over traditional antibiotics.

5. CURRENT LIMITATIONS OF USING PEPTIDES TO TREAT INVASIVE MRSA INFECTIONS

Though AMPs possess multiple desirable properties as therapeutic agents, their ability to be used systemically has been hindered due to a number of different physical limitations. This is problematic, as it pertains to multidrug-resistant staphylococci, because invasive MRSA infections still pose a significant challenge to the healthcare system. Currently available antibiotics fail to treat these systemic infections due to emerging resistance by MRSA to these agents, poor pharmacokinetic profile of many antibiotics (including agents of last resort like vancomycin), and inability to cross cellular membranes of host cells to reach intracellular niches where MRSA colonize and prolong infection (such as macrophages). While there was once great hope that AMPs would be able to overcome the challenges faced by traditional antibiotics, these peptides have significant limitations as well. Peptides cannot be administered systemically for the treatment of invasive MRSA infections for several major reasons including (1) toxicity to host tissues, (2) lack of oral bioavailability, (3) loss of antibacterial activity in the presence of physiological concentration of salts and divalent cations, (4) loss of antimicrobial activity due to serum binding, (5) rapid degradation of peptides by proteases, and (6) inability of peptides to kill MRSA present inside host cells. A more detailed explanation of these drawbacks will be presented in the following paragraphs.

5.1. Toxicity to host tissues

A significant challenge facing researchers trying to develop peptides as novel antibacterials is the fact that many AMPs are toxic to mammalian cells (29). Indeed toxicity to the liver, kidneys, and eyes (for ophthalmic agents) has been reported for many AMPs (40). Additionally, a high degree of toxicity has been observed in mammals when peptides are injected directly into the circulatory system. Furthermore, given the mode of action of several AMPs (including melittin from bees) as nonspecific membrane-disrupting agents, they can attack the integrity of host cells including red blood cells, leading ultimately to hemolysis (31, 40).

The toxicity associated with natural AMPs which are part of the innate immune response/ host defense system are often controlled by various host processes including sequestration of peptides in granules in immune cells, generation of nontoxic propeptides that require enzymatic cleavage to achieve the biologically active peptide, and programmed deactivating mechanisms to limit damage to host tissues (40, 146). However AMPs as therapeutic agents are often given as is (at concentrations higher than those found in the human body); given that many AMPs have a low therapeutic index, this creates problems with toxicity when

higher doses of peptides are administered. Thus one appropriate mechanism to address this issue would be to develop vehicles to transport and release AMPs at the site of infection to minimize toxic side effects to healthy tissues. However, limited success has been observed in this area as it pertains to cationic AMPs (147).

5.2. Limited bioavailability of peptides administered orally or intravenously

Some AMPs (such as mersacidin) are not soluble in water resulting in limited bioavailability of the AMP in host tissues when administered orally or intravenously (28). Though degradation of peptides by proteases or serum binding are one explanation (as discussed in more detail below), another explanation is that AMPs cannot cross membrane barriers present in host tissues (such as the intestinal tract) to reach the bloodstream and ultimately reach the site of infection. This presents challenges in terms of drug delivery and penetration across biological membranes. A publication assessing the effect of the antibacterial peptide PG-1 found the AMP exhibited potent activity against MRSA ATCC 33591 *in vitro* (MIC of 2 μg/mL). However, when mice were subjected to an intravenous dose (4 mg/kg) of PG-1, the peptide concentration diminished rapidly and was not detectable in the plasma after two hours (63). The authors postulated that this most likely was due to limited tissue penetration of PG-1. This hypothesis was confirmed when pharmacokinetic experiments with PG-1 were performed via an intramuscular injection (8 mg/kg) of the peptide in mice; no PG-1 was able to be detected in the plasma even ten minutes after the injection was administered. Thus the peptide was not able to diffuse through the muscular tissue and enter the bloodstream. The researchers concluded that bacteria that concentrated in host tissues following intravenous dosing were most likely not exposed to an effective concentrations of PG-1 to kill the bacteria. One solution to resolve the issue of aqueous solubility of peptides is to incorporate a salt (such as an alkali metal like potassium) into the peptide structure; however, this may compromise the antimicrobial activity of the peptide. Another potential solution would be to design a vehicle that would permit the peptide to be delivered directly to the site of infection but to date no such success has been reported.

5.3. Loss of antibacterial activity due to serum binding

A third limitation of AMPs for systemic applications is, given these molecules are peptides, they tend to bind strongly to proteins in the serum. Human serum albumin is the predominant transport protein present in blood plasma; a recent study demonstrated that the hydrophobic residues present in AMPs have a high affinity (in μM concentration) for binding to drug site II in human serum albumin (42). The binding affinity of peptides to serum proteins reduces the distribution and concentration of the active drug that can reach the site of infection. Drugs that bind strongly to serum proteins (>95% bound) present several issues including requiring a higher concentrations to achieve a therapeutic effect *in vivo*, slow distribution to the target site of infection and issues with elimination of the drug from the host (148, 149). Though increasing the size/concentration of the dose of an AMP given or the frequency of administration would appear a simple solution to this conundrum, this can lead to severe toxicity in host tissues. It has been postulated that the cationic charge of peptides contributes to their binding to tissues and proteins and their overall stability; thus studies have been undertaken to explore modified peptides where these positively-charged

residues were removed. However, this resulted in complete loss of activity, indicating the necessity of these residues to achieve an antibacterial effect (150).

5.4. Loss of antibacterial activity in the presence of physiological concentrations of salts and divalent ions

Additionally, the presence of high concentrations of salts (100 mM NaCl) or monovalent and divalent cations (1–2 mM) in the body (such as in serum/plasma) can interfere with cationic antimicrobial peptides' ability to interact and kill pathogenic bacteria (27, 41, 151). For example, the activity of defensins, weakly antibacterial peptides derived from mammals, have been shown to be negatively affected by increasing salt concentrations (31). Similar results have been reported for other peptides including clavanins, histatins such as P-113, gramicidins, and magainins (33, 150, 152). Several published reports have also mentioned that peptides tested in standard nutrient-rich growth medium were capable of inhibiting bacterial growth at very low concentrations; however when the peptides were tested in solutions/buffers containing increasing concentration of salts or divalent cations (such as 50–100 mM NaCl or $MgCl_2$ to mimic conditions present inside the human body), the antibacterial activities of these peptides were significantly reduced or completely abolished (95). This is problematic for assessing the true potential of AMPs against bacteria given that different culture media (depending on the presence or absence of salts) can produce confounding results (95). Given the presence of such concentrations of salts in the human serum and different regions of the human body where infections can persist, it points to a problem with using AMPs systemically; namely that their antimicrobial activity will most likely be lost due to serum binding.

5.5. Degradation by host and bacterial proteases and bacterial adaptation to minimize peptide effect

Another major limitation of AMPs as systemic agents is the structural integrity of these peptides can be compromised as they are often rapidly degraded by host proteases (particularly those present in the GI tract) (31). In addition to the proteases present in the GI tract, AMPs can be degraded by peptidases present in the blood, as has been shown for other protein drugs (153). This has a profound effect on the pharmacokinetic profile of these peptides, particularly minimizing the quantity of drug available to be absorbed into the bloodstream (once again limiting the use of peptides for systemic applications). Antimicrobial peptides contain basic amino acids in their core structure which makes them susceptible to proteases such as the chymotrypsin-like enzymes (41). Several researchers have tried to address the issue of proteolysis of peptides by introducing covalent modifications to the bonds between the amino acids, insertion or substitution of unusual amino acids (such as D-amino acids which protects peptides from proteolytic degradation), altering the secondary structure of the AMPs, utilizing non-peptide backbones (peptidomimetics), and the creation of prodrug molecules (154–157). However, this has produced mixed results with regards to an increase, decrease, or no change observed in the antimicrobial activity of these AMPs against MRSA and other strains of staphylococci (154, 155, 157). Additionally, the introduction of these modifications, in particular the D-amino acids, comes at a price as these peptides cost more to develop (158). Furthermore, no information is presented regarding their potential toxicity to host tissues, stability in

physiological salt concentrations, serum binding profile, or impact of the modifications on the overall pharmacokinetic profile of these peptides. Thus limited information is available to assess the true potential of these modified AMPs for use systemically.

In addition to rapid degradation by host proteases, the antibacterial effect of AMPs can be suppressed by bacteria that secrete effector molecules that degrade peptides, prevent peptides from interacting with the bacterial cell membrane (blocking their ability to target and enter into the cell), or suppress the antibacterial effect of peptides altogether. An example of this is the staphylokinase protein secreted by *S. aureus* which is capable of binding directly to, and neutralizing, α-defensins secreted by human neutrophils (159). In addition to staphloykinase, *S. aureus* can secrete proteases, including aureolysin, which has been shown to directly interact and breakdown the peptide LL-37 (160). Additionally, given that the cationic moieties of peptides (particularly of defensins, cathelicidins, and their synthetic analogues) play an integral role in interacting with the negatively-charged regions present on the surface of bacterial cells, this electrostatic interaction can be reduced or revered by alterations made to surface components on bacterial cells. For example *S. aureus* can incorporate an l-lysine modification within phosphatidylglycerol or a d-alanine modification to the teichoic acid component of cell walls, reducing the negative charge present in the cell wall (161, 162). These modifications will reduce the affinity and adsorption of AMPs to these bacterial cell membranes. Furthermore, a limited number of *S. aureus* strains have been identified which contain a plasmid (pSK1) encoding a gene (*qac*A) for an efflux pump (163). Though this efflux pump appears to have limited effect on the removal of most natural AMPs present in the human body (such as α-defensin), its identification does raise concern that efflux pumps may be a potential challenge that future synthetic AMPs may have to overcome. Most research, to date, on bacterial adaptation to AMPs has focused on natural peptides derived from humans and animals. More work needs to be employed to understand potential mechanisms of bacterial adaptation to synthetic analogues of these AMPs.

Recent reports have presented several solutions to enhance the stability of antimicrobial peptides to the effect of proteases, limit serum binding, and mitigate the loss of antimicrobial activity in the presence of physiological concentration of salts. Given the simplicity and structural versatility of AMPs, it was thought that simple modifications to the basic structure of the peptide could mitigate stability and pharmacokinetic issues identified with many AMPs. Methods employed included the use of hydrophobic oligopeptide end tags and substitution of tryptophan- and histidine-rich peptides with the amino acid β-naphthylalanine (152, 164). While these techniques provided valuable insight into improvements to enhance the stability and activity of antimicrobial peptides, they did not address or effectively resolve all of the limitations described above.

5.6. Inability to penetrate intracellular compartments to kill *S. aureus*

S. aureus has been shown to be capable of escaping the host immune response to infection by modulating its gene expression in a manner that permits it to reside inside phagocytic cells, including macrophages (165). This action protects *S. aureus* from the effect of many antibiotics (incapable of gaining entry into these intracellular niches) and can lead

to recurring infections in affected patients, including those suffering from osteomyelitis. Though peptides are smaller in size relative to proteins (which should permit their rapid diffusion across biological membranes), their amphipathic nature creates problems in crossing biological membranes, including those present across host cells and in the intestinal tract (153). This observation has been confirmed in several studies including a report published by Brinch *et al* with the peptide NZ2114. While the peptide possessed excellent activity against MRSA *in vitro* (MIC was 4 mg/L), similar to daptomycin and vancomycin, the peptide proved unable to effectively clear MRSA present inside infected THP-1 monocytes (69). Though the authors did not record the actual drug concentration present inside the monocytes, the inability of NZ2114 to effectively enter and survive in the infected monocytes is a plausible explanation. A more recent study utilizing short β-sheet folding synthetic peptides tried to address this issue of intracellular penetration. One of the engineered peptides, Iκ8-all D possessed excellent *in vitro* activity against *S. aureus* (MIC of 3.9 mg/L), demonstrated limited hemolytic activity (less than 10% even at a concentration of 1500 mg/L), was not degraded in the presence of proteases, and produced a 5–6 $\log_{10}$ reduction in *S. aureus* CFU/mL in an infected murine macrophage cell line (166). However none of the assays performed were under true physiological conditions or were confirmed in an animal model. Thus these results, while promising, must be translated into animal studies to confirm these synthetic peptides are in fact capable of eliminating *S. aureus* present in intracellular compartments.

5.7. Additional challenges

Additional challenges pharmaceutical companies and academic research groups will need to overcome with AMPs to translate their *in vitro* potential to actual clinical applications (particular systemically) include addressing issues pertaining to the sensitivity of AMPs to temperature, the economic feasibility to manufacture AMPs at a large-scale, and selecting appropriate packaging material and storage conditions to enhance the shelf-life and stability of the AMPs. As an example of the sensitivity of AMPs to temperature and storage conditions, one study assessing the efficacy of synthetic AMPs for eradication of pathogens causing an ocular infection noted that the AMPs developed were sensitive both to the storage medium and temperature. When the storage temperature was decreased from 23°C to 4°C, the AMP lost complete antimicrobial activity against *S. aureus* (146). This presents a significant problem with regards to maintaining appropriate storage conditions to ensure AMPs do not lose antimicrobial activity prior to administration for treatment.

With regards to the economic feasibility of manufacturing AMPs, they are expensive to manufacture in bulk and there are considerable challenges for small companies and university researchers to develop large enough quantities to test for further applications (29). A single gram of peptide (sufficient for an average daily dose orally or via intravenous injection) may cost upwards of $600 to manufacture (41). This significantly hinders researchers in their pursuit of developing peptides as novel anti-infective agents. Furthermore, several commercial antibiotics, such as vancomycin and erythromycin, still retain more potent activity than most AMPs (MIC values of AMPs are 15–25 fold higher than the antibiotics) *in vitro* and in *in vivo* models (more animals survive after treatment) (167). This points to the argument that conventional antibiotics are still a better option,

currently, for the treatment of invasive infections compared to antimicrobial peptides. Collectively these points raise significant concerns and challenges to utilizing AMPs to treat systemic infections, particularly those caused by multidrug-resistant staphylococci such as MRSA.

6. PEPTIDOMIMETICS – ALTERNATIVES TO AMPs FOR TREATMENT OF *S. aureus* INFECTIONS

The limitations observed with AMPs spurred the development of synthetic mimics of these peptides, the so-called peptidomimetics. Peptidomimetics resemble a peptide structure with the exception that the backbone is not limited to the presence of α-amino acids (158). This non-traditional backbone has a selective advantage over natural peptides in that the unique structure of peptidomimetics is protected from the effect of proteases. An additional benefit, peptidomimetics are less expensive alternatives to manufacture over peptides (41). The mechanism of action of peptidomimetics mirrors that of peptides in that both often target the cell membranes of bacteria though alternative modes of action for specific compounds have been proposed. Different methods have been employed to develop peptidomimetics with antimicrobial activity; one method which led to the development of one of the first peptidomimetics to reach clinical trials utilized Protein Epitope Mimetic (PEM) technology. This technology led to the development of iseganan (IntraBiotics Phamaceutical, Inc.), a synthetic analogue of the AMP protegrin I. It was indicated for use in treating oral mucositis but the peptide failed in clinical trials. It was also tested in a phase III trial as a prophylactic agent to prevent ventilator-associated pneumonia; however the trial was stopped after a higher rate of ventilator-associated pneumonia and death were observed in patients after treatment with iseganan as compared to the control (158). This finding tempered initial expectations that peptidomimetics could overcome the limitations associated with clinical failure of AMPs.

A second technique to develop peptidomimetic agents that has shown great promise is *in silico* design of synthetic mimics of antimicrobial peptides (SMAMPS) which utilize economical synthetic oligomers (158). This effort has led to the development of antimicrobial compounds (termed defensin-mimetics) exhibiting improved toxicity, proteolytic stability, and pharmacokinetic properties relative to AMPs. One of these compounds, brilacidin (PMX-30063), developed by PolyMedix, Inc. (acquired by Cellceutix Corp. in 2013) demonstrated efficacy, even better than vancomycin, against MRSA infections in animal models. Based upon the preliminary studies conducted, brilacidin was proposed to be used as an injectable formulation for several infections caused by *S. aureus*, including the treatment of skin and soft tissue infections. Recently, a phase II clinical trial was completed; this trial demonstrated a high clinical response rate (as compared to daptomycin) in patients treated for acute bacterial skin and skin structure infections caused by MRSA (168). Furthermore, limited toxicity to patients undergoing treatment was reported from this study. These results, combined with previous studies indicating the drug has a low frequency of bacterial resistance, demonstrate the great promise peptidomimetics have as novel, safe alternative antibacterials which can be developed in the near future.

7. ANTIMICROBIAL PEPTIDES AND PEPTIDOMIMETICS IN CLINICAL TRIALS

The development of peptide-based therapies is gaining momentum, evidenced by the increased number of US Food and Drug Administration (FDA)-approved peptide-based therapeutics, which currently exceeds 100 different drugs. Although there are a few antimicrobial peptides (AMPs) and peptidomimetics that have reached various stages of clinical trials for the treatment and prevention of *S. aureus* infections, none have received regulatory approval to date (Table 2). Among the clinically-tested peptides, pexiganan showed the most promising results for the treatment of mildly infected diabetic foot ulcers (DFU) in two clinical trials (NCT00563433 and NCT00563394). Pexiganan proved as effective as oral ofloxacin for the treatment of mildly infected DFU though with fewer side effects and reduced likelihood of bacterial resistance developing (169). After several years and several successful clinical trials, pexiganan did not receive FDA approval. However, topical pexiganan cream 0.8% is currently being tested again in two Phase III clinical trials for the treatment of mild infections of DFU (NCT01590758 and NCT01594762).

7.1. The demand for topical treatments for infected DFU

The damaging role of *S. aureus* in DFU is well recognized since it is the most frequently isolated microorganism in infected DFU patients. Additionally, infections caused by multidrug-resistant *S. aureus* strains are associated with the worst patient outcomes and result in more frequent amputation of limbs (130–135). The increasing prevalence of infected DFU and the increasing resistance among associated *S. aureus* isolates highlights the dearth of adequate antimicrobials to treat DFU infections. Therefore, there is an urgent need to develop new topical therapies capable of treating and promoting the healing of infected DFU. The estimated healthcare-associated costs of $15 billion annually for treatment of chronic wounds (170) such as DFU should create an economic incentive for pharmaceutical companies to invest in and develop AMPs capable of filling this unmet medical need for new antimicrobials. Additionally, independent of their antimicrobial effect, the recognized anti-inflammatory, immunomodulatory, and antibiofilm activity of certain AMPs should be a major advantage in the topical treatment of *S. aureus* skin infections in general and of infected non-healing DFU, in particular.

7.2. Future of clinical applications of AMPs

The major impediments to the translation of AMPs to the clinic thus far appear to center heavily on the stringent clinical trial designs required by regulatory agencies. Additionally the lengthy waiting process which follows the completion of trials followed by failure to receive FDA approval has slowed the progression of AMP development into the healthcare setting. This issue is further compounded by the fact that the recent FDA “reboot” pledge (171) to encourage the development of new antibiotics is unlikely to entice pharmaceutical companies to invest in this long and risky process of development of new antimicrobial drugs, including AMPs.

It is difficult to predict the possibility of AMPs receiving approval for use against *S. aureus*-related infections in the next few years. However, the low number of AMPs that are

currently in clinical trials (Table 2) would suggest that the likelihood of an AMP receiving approval for use against *S. aureus* infections will not occur in the immediate future. Though AMPs as antibacterial agents may not make it to the clinical setting soon, work must continue to address their limitations and identify new avenues to pursue to propel their development as novel therapeutic agents.

8. POTENTIAL FUTURE APPLICATIONS FOR PEPTIDES IN TREATMENT OF *S. aureus* INFECTIONS

Though limited success has been achieved thus far in the development of AMPs for clinical use as antibacterials, they still hold great promise for use as therapeutic agents to treat staphylococcal infections. Their unique advantages over natural product antibiotics combined with a better understanding of their limitations has permitted a more focused strategy to be initiated to identify future applications for AMPs currently in development. Among their potential applications include (1) as topical ointments to treat skin infections and enhance wound healing, (2) use in combination with conventional antibiotics or peptides to produce a synergistic effect against *S. aureus*, (3) as immunomodulatory agents to clear bacterial infections, and (4) as decolonizing agents in both the healthcare and community settings. We will briefly touch upon the use of AMPs to address these strategies against MRSA in the subsequent paragraphs.

8.1. Topical ointments to treat skin infections

S. aureus is the most common bacterial pathogen linked to patients with skin and soft-tissue infections (SSTIs) (175). More than half (58%) of all SSTIs treated in the United States alone were caused by *S. aureus* per one epidemiological study of one national health care system (176). The Infectious Diseases Society of America recommends treatment of moderate to severe skin infections caused by MRSA to involve the combination of incision and drainage of the affected region with administration of empirical antibiotics (including mupirocin) (177, 178). However, highly-resistant strains of MRSA, including to mupirocin have been documented presenting significant concern that such therapies may be rendered ineffective in the future (22, 179, 180).

Antimicrobial peptides have the potential to be strong allies in the battle against MRSA skin infections given their potent anti-MRSA activity and low frequency of bacterial resistance emerging. Additionally, AMPs have been shown to possess anti-inflammatory properties and to enhance wound healing, both qualities which are critical for treatment of patients affected by a skin infection. Development of AMPs for topical applications has several appealing qualities including (1) direct delivery to the site of infection (thus avoiding the necessity of a vehicle or carrier as with systemic applications), (2) high local tissue concentration of the drug to be achieved through aggressive dosing combined with AMPs rapid bactericidal activity (permitting rapid clearance of an infection), (3) using a subinhibitory concentration of AMPs in combination with traditional antibiotics to achieve a synergistic effect (limiting potential irritation and toxicity to the skin), and (4) avoid issues pertaining to serum binding and proteolysis which can render AMPs ineffective (particularly when used systemically). Furthermore, toxicity to host issues (which is a significant problem for AMPs developed for

systemic applications) can be mitigated by altering the dosage, frequency of administration, and formulation of the final product.

The success of AMPs developed for topical applications which have reached human clinical trials supports the proposition of developing AMPs for the treatment of MRSA skin infections. Among this list includes AMPs such as pexiganan, MBI 594AN, and D2A21. Pexiganan is a synthetic analogue of maganin which showed broad-spectrum activity against over 3000 clinical isolates of bacteria *in vitro* (including MRSA and VRSA) and, as stated earlier, achieved the same cure rate (85–90%) and wound healing as the control antibiotic ofloxacin in the treatment of patients with diabetic foot ulcers in a Phase III clinical trial (40, 181). MBI 594AN, an indolicidin-like peptide was developed as a topical ointment for the treatment of acne (primarily caused by the bacterium *Propionibacterium acnes*); in a Phase IIb clinical trial, this ointment produced a statistically significant reduction in the size of inflammatory acne lesions on the skin when compared to the control vehicle (40). D2A21 is another peptide under development as a topical antibiotic that has shown potent activity against MRSA *in vitro* (MICs between 0.25 – 4 μg/mL) (182); when tested in a rat model (against *Pseudomonas aeruginosa*), D2A21 demonstrated potential for use in the treatment of both skin infections (183) and burn wounds (184). Though none of these AMPs have reached the market yet, their success in *in vivo* animal models and human clinical trials, lends credence to the potential for AMPs to be used in the treatment of MRSA skin infections.

The rapid and potent antibacterial activity of peptides also has potential to limit the severity of skin infections by preventing bacterial migration into systemic circulation leading to invasive MRSA infections. Invasive MRSA infections are of particular concern as they can lead to pneumonia, sepsis, and eventually death (185). Thus development of AMPs as topical ointments to treat MRSA skin infections is a viable therapeutic strategy that warrants further investigation for researchers.

8.2. Use as decolonizing agents

A second potential application for AMPs against MRSA is using them as decolonizing agents. MRSA has shown the ability to colonize body surfaces including the skin, nares, and mucosal membranes (186, 187). Additionally, MRSA is capable of colonizing medical devices, in particular catheters, which can have serious consequences to many patients including those undergoing surgery, requiring dialysis, and patients suffering from atopic dermatitis (MRSA colonization leads to more severe skin lesions developing) (187). Breaches in the skin, a first-line of defense against bacterial infection, can permit the pathogen to gain access to the bloodstream and result in potentially fatal diseases, including sepsis. One study demonstrated that 19–25% of patients who were colonized with MRSA in the healthcare setting subsequently developed a MRSA infection (186). Another study found 29% of patients colonized with MRSA in the healthcare setting developed a MRSA infection within 18 months after being discharged from the hospital (188). Such infections have been linked to lengthier hospital stays and higher costs due to infection control guidelines (including patient isolation/decolonization) and therapeutic intervention required to treat the disease (189). This is of particular concern to the pediatric and geriatric

populations who are more susceptible to infection (with more severe morbidity) due to a less developed/weaker immune system. One way to deal with this challenge is to utilize agents to reduce MRSA colonization of human tissues, reducing the transmission of MRSA between people and decreasing the likelihood of an infection from developing.

Antibiotics, as decolonizing agents, have been shown to have a profound effect on decreasing MRSA colonization of host tissues (187). Mupirocin has been the drug of choice as a decolonization agent for many years (190). However, as highlighted earlier, strains of MRSA exhibiting moderate to high level of resistance to mupirocin have emerged. Of even greater concern are isolates of the predominant strain of MRSA (USA300) in the United States that have been documented to exhibit resistance to mupirocin (191). Mupirocin-resistant strains will naturally result in decolonization failure when this antibiotic is used. Thus alternative decolonization agents to mupirocin are critically needed.

The combination of potent antibacterial activity with low level of bacterial resistance emerging makes AMPs an attractive alternative to antibiotics as decolonizing agents. Successful experiments performed in animal models and clinical trials have given further support to the use of AMPs as decolonizing agents. A phase IIIa clinical trial with the peptide MBI-226 (Omiganin), developed by Migenix, demonstrated a 40% reduction in MRSA catheter colonization (31). This result lends further credibility to the usage of AMPs as decolonizing agents in addition to alternative topical antibiotics for the treatment of skin infections and immunomodulating agents for the treatment of MRSA infections.

8.3. AMPs for use in combination therapy

The development of novel antimicrobials has not been able to keep pace as the rate of bacterial resistance to antibiotics has continued to rise. Identification of new antimicrobials for use as single agents is a challenging and costly venture that has achieved very limited success in the past four decades. New antimicrobials which receive regulatory approval are often modified versions/derivatives of previously discovered antibiotics with more broad-spectrum activity (such as 4^{th}- and 5^{th}-generation cephalosporins). Though identifying novel lead antimicrobial compounds with a unique mechanism of action is one potential treatment strategy for bacterial infections, other methods have been employed. One of these methods which has observed considerable success is the use of combination therapy with two or more antibiotics (192). Using two antimicrobials together has multiple benefits including the usage of lower concentrations of each drug thus limiting potential issues associated with toxicity to host tissues (as higher concentrations of each drug given alone may trigger side effects at the effective therapeutic dose) and protection from bacterial resistance emerging rapidly to both antimicrobials given together. Currently, combination therapy involving the antibiotics vancomycin and rifampin in addition to sulfamethoxazole and trimethoprim have been successfully utilized against MRSA (193). A similar strategy employing an AMP in combination with a conventional antibiotic has received more attention in recent years (79, 95, 194).

There are multiple characteristics to look for in a suitable partner to test with a particular antimicrobial for combination therapy. AMPs possess two important characteristics that have been identified as important factors for using an agent in combination with another

antibiotic including a low frequency of bacterial resistance and an antimicrobial that can rapidly kill the bacteria of interest (193). The fact that AMPs target different biological targets from most traditional antibiotics ensures they can complement the efforts of these conventional therapeutic agents to eradicate difficult pathogens, like MRSA. Several studies have highlighted a synergistic relationship being present when an AMP is combined with a natural product antibiotic. A study performed by Midorikawa *et al*, compared the efficacy of different AMPs produced by keratinocytes (including human β-defensin-1 (hBD1), hBD2, hBD3, and CAP18) both as stand-alone agents and in combination with one another (95). Interestingly, they found that when β-defensins were combined with CAP18, a synergistic relationship was observed against MRSA. Furthermore, when hBD3 or CAP18 were combined with methicillin at subinhibitory concentrations, a synergistic relationship was observed against ten MRSA strains.

Research conducted by our lab has identified salt-resistant synthetic peptides (RRIKA and RR) that exhibit potent activity *in vitro* and *in vivo* (in a murine skin infection model) against MRSA (79, 194). These peptides were found to be nontoxic to mammalian cells at concentrations 4- to 8-fold higher than their MICs against MRSA and exhibited limited hemolysis against human red blood cells (up to a concentration of 300 μM). Even more interesting was the fact that when these peptides were combined with the cell wall-disrupting antibiotic lysostaphin, a synergistic relationship was observed (with a fractional inhibitory concentration (FIC) value of 0.26, identified via the checkerboard assay). Furthermore, an additive effect was observed when these two peptides were combined with conventional antibiotics (vancomycin and linezolid) used to treat MRSA infections. This lends further support to the potential use of AMPs as combinatory agents with antibiotics against MRSA, particularly with skin infections.

8.4. Use as immunomodulatory or immunostimulatory agents

Naturally-occurring peptides, such as the defensins in humans, have been shown to play an important role in the immune response generated against invading pathogens. These molecules play an important role in innate immunity as they are often constitutively expressed or produced in response to microbial virulence factors such as lipopolysaccharide (in Gram-negative bacteria). However, studies have also shown that, in the presence of certain cytokines, leukocytes (including human natural killer cells, B cells, and γδT cells) can be induced to produce peptides such as α-defensins indicating they may play a vital role as effector molecules in adaptive immunity (195, 196). While defensins have been shown to possess potent antibacterial activity at low concentrations against *S. aureus* (196, 197), it points to a potential alternative use for peptides – as modulators or stimulators of the host immune response to clear invading pathogens. Thus peptides possessing limited or no antimicrobial activity but with immunostimulatory properties (to selectively upregulate the innate immune response to an infection without overstimulation of pro-inflammatory mediators) have potential use as therapeutic agents against *S. aureus* (particular for clearance of intracellular infections). This action can be combined with a subtherapeutic dose of a traditional antibiotic to achieve a two-fold attack on the bacterial infection – direct action against the pathogen (achieved by the antibiotic) combined with stimulation of the immune response (by the peptide) to clear the infection. Inimex Pharmaceuticals Inc.

successfully demonstrated this strategy using a peptide (to enhance the immune response) in combination with a traditional antibiotic that worked in a murine *S. aureus* infection model to significantly reduce the bacterial load *in vivo* (40).

The immunomodulatory effect of AMPs is promising because it involves a different method of use over antibacterial action. One such peptide, LL-37 which has low to moderate antibacterial activity against *S. aureus*, was shown to be capable of reducing the bacterial load of *S. aureus* in a mouse model. This effect was postulated to be linked to LL-37's ability to modulate the host immune response. LL-37 plays a role in the induction of chemokine production (IL-8, monocyte-chemoattractant proteins 1 and 3) necessary to attract immune cells including monocytes, neutrophils, and dendritic cells to the site of infection; thus it was proposed that these immune cells (induced by LL-37) were responsible for helping to clear the infection (151).

The immunomodulatory function of AMPs (such as IDR-1018) and AMP fragments (including HB-107) lacking antibacterial properties, has also been shown to accelerate wound repair (198), including wounds infected with *S. aureus* in animal models (199). Additionally, IDR-1018 was shown to possess both immunomodulatory activity (inducing the release of chemokines) while simultaneously suppressing a pro-inflammatory immune response (200). Controlled stimulation of the innate immune response by AMPs is critical; triggering the innate immune response often leads to excessive production of pro-inflammatory cytokines and effector molecules that can cause severe tissue damage or even lead to sepsis, a disease that kills 140,000 people every year in North America (41, 201).

9. CONCLUSION

Multidrug-resistant infections, in particular those caused by *S. aureus*, are a daunting public health challenge that requires serious attention. The diminishing utility of current antibiotics in the face of rising bacterial resistance further underscores the urgent need for the development of alternative therapeutic options and strategies to conventional antibiotics. Antimicrobial peptides present a strong potential ally in the battle against bacterial infections given their complex mode of action makes bacterial resistance to AMPs unlikely to rapidly emerge. Though these peptides possess several limitations which need to be addressed to use them in systemic applications (including stability in physiological conditions and limited ability to penetrate intracellular compartments where *S. aureus* tends to escape the host immune response), success in animal studies and human clinical trials supports the usage of AMPs as decolonizing agents and topical agents (either alone or in combination with conventional antibiotics) for the treatment of *S. aureus* skin and soft-tissue infections. Additionally, given antimicrobial peptides have the ability to modulate the host immune response, their potential use as immunomodulatory agents to stimulate the host immune response to clear an infection caused by multidrug-resistant bacteria presents another exciting avenue for researchers to pursue for the future. Though regulatory approval of AMPs for antibacterial application has yet to be achieved, the number of natural and synthetic peptides currently in clinical trials, and under development, presents hope that these compounds will be important clinical allies in the future for the treatment of bacterial infections.

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Table 1 -

Antimicrobial peptides and peptidomimetics with biological activity against *S. aureus*.

Peptides	Source	Sequence	MIC	Toxicity	References
Indolicidin	Bovine neutrophil granules	ILPWKWWPWRR	5–16 μg/ml	LD_{50} of indolicidin in MT-2 cells is 33 μg/ml	(43–45)
Omiganan (MX-226)	Synthetic cationic peptide derived from indolicidin	ILRWPWWPWRRK	16–32 μg/ml	1% omiganan gel resulted in no adverse local or systemic effects in repeated-dose (14-day and 28-day in rat and rabbit models)	(46–50)
LL-37	Human neutrophil granules, epithelial cells (skin, lung, gut, mammary gland and epididymis)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	1–8 μg/ml	Low dose (100 μg /kg of body weight) did not induce observable toxicity but a high dose (3,000 μg/kg) resulted in adverse effects in rats	(51–53)
Novispirin G10	Variant of ovispirin-1 from sheep	PKNLRRIIRKGIHIIKKYG	4–12 μg/ml	2.5% hemolysis of human erythrocytes at 80 μg/ml	(54, 55)
Temporin A	Frog (*Rana ornativentris*) skin	FLPLASLFSRLL	5 μM	Cytotoxic effect not observed in keratinocytes below 10 μM	(56, 57)
Iseganan (IB-367)	Derivative of protegrin, from porcine neutrophils	RGGLCYCRGRFCVCVGR	4 μg/ml	Intraperitoneal injection of IB-367 at a dose of 1mg/kg body weight caused no systemic toxicity in rats	(58–60)
Brilacidin (PMX-30063)	Defensin-peptide mimetic	N.A	1–2 μg/ml	EC_{50} for RBCs is greater than 500μg/ml	(61, 62) PolyMedix Inc.
Protegrin-1 (PG-1)	Porcine leukocytes	RGGRLCYCRRRFCVCVGR	1–2 μg/ml	42% hemolysis at 100μg/ml	(63, 64)
HB1345	Synthetic lipohexapeptide	Decanoyl- KFKWPW	0.5–1 μg/ml	Not available	Helix Biomedix
Pexiganan acetate (MSI-78)/ Magainin peptide	Skin of the African clawed frog (*Xenopus laevis*)	GIGKFLKK AKKFGKAF VKILKK	16–64 μg/ml	250 μg/ml are necessary to induce 100% hemolysis	(65, 66)

Peptides	Source	Sequence	MIC	Toxicity	References
Plectasin	Defensin-like peptide from saprophytic ascomycete *Pseudoplectania nigrella*.	GFGC1NGPWDEDDMQC2HNHC3KSIKGYKGGYCAKGGFVC2KC3Y	16–32 μg/ml	Hemolytic dose EC_{50} for human erythrocytes is 400 μg/ml	(67)
NZ2114	Derivative of plectasin	N.A	2–4 μg/ml	Less than 1% cytotoxicity observed in THP-1 cells at 128μg/ml	(68, 69)
Fowlicidin-1 (6–26)	An α-helical cathelicidin from chicken	RVKRVWPLVIRTVIAGYNLYRAIKKK	1–2 μM	Hemolytic dose EC_{50} for human erythrocytes is ~40 μM	(70)
hLF1–11	Derivative of Lactoferrin (LF), an iron-binding glycoprotein present in various secretory fluids such as milk, saliva, tears, and mucosal secretions	GRRRRSVQWCA	4–16 μM	No toxicity observed in doses up to 10 mg/kg body weight.(at least 10,000 times higher than the dose proposed for treatment in humans)	(71–75)
Lytixar (LTX-109)	Synthetic peptidomimetic	N.A	2–4 μg/ml	Toxicity not observed in topical application of 5% LTX-109 for 14 days in rats and mini-pigs	(76–78) Lytix Biopharma
RR	Synthetic peptide	WLRRIKAWLRR	8–32 μM	Less than 10% hemolysis noticed at 300 μM and toxicity not observed in HeLa cells up to 64 μM	(79)
Nisin	Lantibiotic from *Lactococcus lactis*	N.A	2–16 μg/ml	4.93% hemolysis of human erythrocytes was noticed at 2.5 μg/ml	(80–82)
RRIKA	Synthetic peptide	WLRRIKAWLRRIKA	2–4 μM	Less than 10% hemolysis noticed at 300 μM and toxicity not observed in HeLa cells up to 32 μM	(79)
MU1140	22-amino acid bacteriocin from *Streptococcus mutans*	N.A	8–32 μg/ml	Not available	(83, 84)

Peptides	Source	Sequence	MIC	Toxicity	References
Epinecidin-1	Peptide from fish (*Epinephelus coioides)*	CFHIIKGLFHAGKMIHGLVTRRRHGV	6.25 μg/ml	Epinecidin-1 at 2 μg/ml caused lysis of RAW264.7 cells	(85–87)
Peptide 4	Cyclic D,L-α-peptide	KQRWLWLW	3 μg/ml	Toxicity not observed in mice at the maximum tested dose of 12 mg/kg with intra-peritoneal injection	(88)
Pardaxin (GE33)	Red Sea flatfish (*Pardachirus marmoratus)*	GFFALIPKIISSPLFKTLLSAVGSALSSSGGQE	6.25 μg/ml	IC_{50} for HeLa cells was found to be 12.36 μg/ml	(89–91)
DASamP1	Synthetic peptide	FFGKVLKLIRKIF	3.1 μM	50% red blood cells lysed at 25 μM	(92)
Human β-defensin 3 (hBD3)	Human keratinocytes, airway epithelial cells	GIINTLQKYYSRVRGGR	4–8 μg/ml	50% of HepG2 and MDCK cells showed toxicity to hBD3 at 25–50 μg/ml	(93–97)
Lysostaphin	Antibacterial enzyme from *Staphylococcus simulans*	Zinc-containing metalloenzyme of 27 kD	0.002–0.032 μg/ml	50% of normal human epidermal keratinocyte cells (NHEK) are toxic at 1.6 g/L	(98–100)
HHC-10	Synthetic peptide	KRWWKWIRW	1.5–3μM	Very low hemolysis (less than 20%) of human erythrocytes was noticed at 188 μg/ml	(101)
HHC-36	Synthetic peptide	KRWWKWWRR	1.5–3μM	Very low hemolysis (less than 20%) of human erythrocytes was noticed at 188 μg/ml	(101)

Table 2 -

Antimicrobial peptides against *S. aureus* in clinical trials.

Peptide name	Sequence/description	Indication	Phase	Clinical trial identifiers	Ref
Pexiganan (MSI-78)	GIGKFLKKAKKFGKAFVKILKK	Infected Diabetic Ulcers	III	NCT00563433 NCT00563394 NCT01590758 NCT01594762	(169)
Iseganan (IB-367)	RGGLCYCRGRFCVCVGR	Ventilator-associated Pneumonia	III	NCT00118781	(172)
Omiganan (MBI 226)	ILRWPWWPWRRK	Topical Skin Antisepsis and Preventing Local Catheter Site Infections and	III	NCT00608959 NCT00231153	(173)
Lytixar (LTX-109)	peptidomimetic Arg-Tbt-Arg-NH-EtPh (arginine-tertbutyl tryptophan-arginine-phenylethan)	Gram-positive Skin Infection and MRSA nasal colonization	II	NCT01223222 NCT01158235 NCT01803035	(77)
Brilacidin (PMX-30063)	peptidomimetic defensin-mimetic	*S. aureus* skin infection	II	NCT01211470	(174)