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Bacterial Evasion of Host Antimicrobial Peptide Defenses

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SUMMARY

Antimicrobial peptides (AMPs), also known as host defense peptides, are small naturally occurring microbicidal molecules produced by the host innate immune response that function as a first line of defense to kill pathogenic microorganisms by inducing deleterious cell membrane damage. AMPs also possess signaling and chemoattractant activities and can modulate the innate immune response to enhance protective immunity or suppress inflammation. Human pathogens have evolved defense molecules and strategies to counter and survive the AMPs released by host immune cells such as neutrophils and macrophages. Here, we review the various mechanisms used by human bacterial pathogens to resist AMP-mediated killing, including surface charge modification, active efflux, alteration of membrane fluidity, inactivation by proteolytic digestion, and entrapment by surface proteins and polysaccharides. Enhanced understanding of AMP resistance at the molecular level may offer insight into the mechanisms of bacterial pathogenesis and augment the discovery of novel therapeutic targets and drug design for the treatment of recalcitrant multidrug-resistant bacterial infections.

Keywords

antimicrobial peptide; antibiotic resistance; bacterial pathogenesis; bacterial resistance; cathelicidin; defensin; host defense peptide; infectious disease; innate immunity; invasive disease; mechanism of action; mechanism of resistance; membrane modification; membrane permeability

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INTRODUCTION

Antimicrobial peptides (AMPs) are small (<10 kDa) soluble host defense peptides that play an important role in the mammalian innate immune response, helping to prevent infection by inhibiting pathogen growth on skin and mucosal surfaces and subsequent dissemination to normally sterile sites. These natural antibiotics are produced by many cell types including epithelial cells, leukocytes (neutrophils, macrophages, dendritic cells and mast cells), platelets, endothelial cells and adipocytes in response to tissue damage or infectious stimuli, and are found in body fluids and secretions including saliva, urine, sweat, and breast milk. To date, more than 2,000 AMPs have been identified from a wide variety of organisms including bacteria, insects, plants, amphibians, birds, reptiles and mammals including humans (1, 2). Whereas prokaryotic AMPs are produced as a competitive strategy to facilitate the acquisition of nutrients and promote niche colonization (3), AMPs produced by higher organisms are generally conceived to carry out immune defense functions. In humans, the principal AMPs are hydrophobic molecules composed of ~10–50 amino acid residues with a net positive charge, which exhibit varying degrees of broad-spectrum bioactivity against Gram-positive and Gram-negative bacteria, fungi, protozoan parasites, and certain enveloped viruses (4, 5). AMPs may be expressed constitutively or induced in response to infection (e.g. pro-inflammatory cytokines, toll-like receptor (TLR) signaling) (6), and are commonly produced as pro-peptides that undergo subsequent proteolytic processing to the mature bioactive peptide (7). AMPs with central roles in host defense are active at micromolar to nanomolar concentrations and facilitate microbial killing through perturbation of the cytoplasmic membrane (8). Several important human pathogens display significant resistance to AMPs, which appears to play a key role in their potential to produce serious invasive infections.

AMPs can be classified into four main groups according to their secondary structure: 1) α-helical peptides, 2) β-sheet peptides, 3) loop peptides, and 4) extended peptides (1, 9). The two major AMP families in mammals are the cathelicidins and the defensins (Table 1). In their mature form, cathelicidins are often α-helical cationic AMPs that do not contain cysteine residues. LL-37 is the sole human cathelicidin (10). Defensins are β-sheet-stabilized peptides classified as either α- or β-defensins according to the pattern formed by three disulphide bridges. α-defensins are primarily produced by neutrophils and intestinal Paneth cells, while β-defensins are expressed by epithelial tissues in the respiratory, gastrointestinal and urinary tracts (11, 12). Mammalian defensins produced by human epithelial and immune cells are cysteine-rich peptide ~30–40 amino acid residues in length (13). Humans produce six α-defensins: HNP 1-4 are found in the azurophilic granules of neutrophil granulocytes (14), while human α-defensins HD-5 and HD-6 are expressed in Paneth cells located in the small intestine (15) and female urogenital tract (16) (Table 1). Six human β-defensins, HBD-1 through HBD-6, have been identified and are expressed by epithelial cells, monocytes, macrophages and dendritic cells (11, 17). Cathelicidins are found in skin cells, gastrointestinal cells, neutrophils and myeloid bone marrow cells (18) (Table 1). Activated platelets produce additional groups of cationic chemokine-related AMPs called thrombocidins and kinocidins (19-21).

These prototypical AMPs have a net positive charge to facilitate interaction with the net negative charge of bacterial surfaces (22). While cationic peptides comprise the largest class of AMPs, certain anionic peptides such as dermcidin produced by eccrine sweat glands, also contribute to host epithelial defense (23). In addition to charge, other factors influencing AMP spectrum and mechanism of action include size, amino acid composition, structural conformation, amphipathicity, and hydrophobicity (24). A primary mechanism of AMP action is through electrostatic interaction with the anionic phospholipid headgroups in the outer bacterial cytoplasmic membrane or cell wall components (22, 25). Upon penetration of the outer membrane or cell wall, AMP insertion into the cytoplasmic membrane causes membrane rupture and cell death (11).

Three general modes of AMP action have been proposed to explain the membrane disruption: 1) the “barrel-stave” mechanism where AMPs directly integrate into the target membrane forming membrane-spanning pores (26); 2) the toroidal-pore mechanism where AMPs form membrane-spanning pores with intercalated lipids inducing a curvature in the membrane (27); and 3) the “carpet” mechanism where AMPs at high concentration accumulate on the cell surface and dissolve the cell membrane in a detergent-like manner without forming membrane-spanning pores (28). In addition to cell membrane perturbation, some AMPs may exert downstream antimicrobial effects by inhibiting the bacterial DNA, RNA, or protein synthesis machinery or biosynthesis of cell wall components (29, 30). Nisin, an AMP commonly used in the food industry as a preservative, is a member of the bacteriocin or lantibiotic family of AMPs that inhibits the biosynthesis of teichoic acid (TA) and lipoteichoic acid (LTA) in Gram-positive bacteria (31). Another bacteriocin, mersacidin, inhibits cell wall peptidoglycan biosynthesis and is active against methicillin-resistant *Staphylococcus aureus* (32). Some eukaryotic defensins target the lipid II biosynthesis pathway, an essential component of peptidoglycan, to inhibit cell wall biosynthesis. Several AMPs inhibit nucleic acid biosynthesis including buforin II (33), indolicidin (34), and puroindoline (35). Human neutrophil peptide 1 (HNP-1), also known as human α-defensin 1, inhibits cell wall, DNA and protein synthesis (36).

Genetic animal models have established an essential role for AMPs in the innate immune system. For example, mice deficient in the murine cathelicidin (mCRAMP) suffer more severe necrotic skin lesions than wild-type (WT) littermates following subcutaneous infection with *Streptococcus pyogenes* (group A *Streptococcus*; GAS), a Gram-positive human pathogen (37, 38). GAS are killed less efficiently by whole blood and mast cells isolated from mCRAMP knockout mice (37, 38), and *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) proliferate better within macrophages of mCRAMP knockout mice (39). Cathelicidin-deficient mice are likewise more susceptible to *Escherichia coli* urinary tract infection (40), meningococcal septicemia (41), *Pseudomonas aeruginosa* keratitis (42), *Klebsiella pneumoniae* lung infection (43) and *Helicobacter pylori* gastritis (23254369), while mice deficient in β-defensin production show impaired defense against *P. aeruginosa* (44) or *Fusarium solani* keratitis (45). In gain-of-function analyses, transgenic mice overexpressing porcine cathelicidin were more resistant to bacterial skin infection (46), while transgenic expression of the human defensin-5 in mouse Paneth cells provided enhanced defense against *S*. Typhimurium enteritis (47).

Beyond their direct antimicrobial activities, AMPs including cathelicidins have also been reported to modulate cytokine production, apoptosis, functional angiogenesis, or wound repair by stimulating keratinocyte migration and proliferation (48-50). Serving as an important link between the innate and adaptive immune system, AMPs may induce the expression of cytokines and chemokines (51, 52), exert direct chemotactic action on neutrophils, macrophages, immature dendritic cells, mast cells, monocytes, and T lymphocytes (53-55), and stimulate histamine release from mast cell to promote neutrophil migration to the site of infection (56).

The resistance mechanisms employed by commensals or microbial pathogens to combat AMPs have been intensively studied over the past two decades. This chapter highlights current information on the direct and indirect mechanisms of action used by human pathogenic bacteria to counteract AMPs, including surface charge alteration, external sequestration by secreted or surface-associated molecules, energy-dependent membrane efflux pumps, peptidase degradation, and the downregulation of AMP expression by host cells. Perturbation of these AMP resistance mechanisms may impair bacterial colonization capacity and reduce virulence in animal infection models. Understanding the molecular mechanisms of AMP resistance may identify novel targets for intervention in difficult to treat bacterial infections.

BACTERIAL AMP RESISTANCE MECHANISMS

Bacterial Surface Charge Modification Increases AMP Resistance

The cationic nature of human AMPs such as defensins and cathelicidin LL-37 provide an electrostatic affinity for bacterial cell surfaces, which are composed of negatively charged hydroxylated phospholipids including phosphatidylglycerol (PG), cardiolipin (also known as diphosphatidylglycerol), and phosphatidylserine (57). In contrast, mammalian and eukaryotic cell membranes contain neutral lipids (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin) and sterols (cholesterol, ergosterol), and carry a net neutral charge that allows selectivity of AMPs for the mostly anionic bacterial cell membranes (3, 57). The amphipathic structure of AMPs resulting from the separation of charged or polar and hydrophobic moieties within the molecule enables their integration into the lipid bilayer of Gram-positive and Gram-negative bacteria, fungi, or viruses, and the formation of destabilizing transmembrane pores that induce cell rupture and death (58, 59).

While Gram-positive bacteria lack an outer membrane, AMP access to the cytoplasmic membrane is inhibited by a thick peptidoglycan-containing cell wall cross-linked with polymers of TA or LTA. In Gram-negative bacteria, AMPs must traverse the outer membrane envelope composed of negatively charged lipopolysaccharide (LPS; up to 70% of the outer membrane) (60), and the periplasmic space beneath the outer membrane, which contains a thin peptidoglycan matrix. Surface-associated proteins and large capsular polysaccharides also hinder AMP access to the cytoplasmic membrane.

One common AMP-resistance strategy used by Gram-positive and Gram-negative bacteria is to increase their net positive surface charge through modification with cationic molecules, resulting in the electrostatic repulsion of cationic AMPs, thus preventing access to and

disruption of the cytoplasmic membrane (Table 2). Several Gram-negative bacteria reduce the net negative charge of LPS lipid A through the addition of 4-amino-4-deoxy-$_L$-arabinose ($_L$-Ara4N), phosphoethanolamine (pEtN), or palmitoyl groups (61). Lipid A is negatively charged and consists of two glucosamine units with free phosphate groups linked to four or more acyl chains (62). Lipid A acylation coordinated by the PhoPQ regulatory system masks the negative surface charge in Gram-negative human pathogens such as *E. coli*, *Salmonella* spp., *Yersinia enterocolitica*, *Haemophilus influenzae*, *K. pneumoniae*, and *Legionella pneumophila* (3, 63). Some Gram-positive pathogens alter their surface charge through the modification of TAs composed of linear anionic glycopolymers of polyglycerol phosphate and polyribitol phosphate linked by phosphodiester bonds. LTAs are non-covalently inserted into the cell membrane with a glycolipid anchor, while wall teichoic acids (WTAs) are covalently attached to the peptidoglycan cell wall by a glycosidic bridge (64, 65). TAs play important roles in bacterial virulence, the adherence and invasion of host cells, biofilm formation (65), antimicrobial resistance (66-68), and activation of the immune response (69, 70). The $_D$-alanylation of teichoic acids by the *dlt* operon and integration of $_L$-lysine into PG by membrane protein multipeptide resistance factor (MprF) are common strategies employed by Gram-positive bacteria to reduce the negative surface charge and enhance AMP resistance (65, 71-73). $_D$-alanylation of TAs is only known to occur in the bacterial Firmicutes phylum (65).

$_D$-alanylation of Cell Wall Teichoic Acids—*S. aureus*, a major Gram-positive human pathogen, is the etiologic agent of abscesses, cellulitis, osteomyelitis, septic arthritis, septicemia and endocarditis. *S. aureus* resists killing by human AMPs through the $_D$-alanylation of cell wall TA. Incorporation of $_D$-alanyl esters into the cell wall by the action of four proteins encoded by the *dltABCD* operon exposes a positively charged amino group, reducing the net negative charge of TAs, and diminishing the electrostatic attraction between cationic AMPs and the bacterial cell envelope (66-68, 72, 74, 75) (Fig. 1A). $_D$-alanine is activated by $_D$-alanyl carrier protein ligase (Dcl; encoded by *dltA*) and delivered to $_D$-alanine carrier protein (Dcp; encoded by *dltC*) with assistance from chaperone protein DltD (encoded by *dltD*). The putative transmembrane protein DltB (encoded by *dltB*) is thought to facilitate transfer of the $_D$-alanyl-Dcp complex across the cytoplasmic membrane (65). The $_D$-alanylation of TA is also dependent upon environmental factors such as temperature, pH and salt (e.g. NaCl) concentration (76, 77). Transcriptional regulators of TA $_D$-alanylation have been identified for several species, including *Bacillus subtilis* (global transcriptional regulators AbrB and Spo0A) (78), group B *Streptococcus* (GBS; two-component system DltRS) (79), and *S. aureus* (global regulators Agr and Rot, two-component system ArlRS) (20). In a recently proposed model, the increased density of the peptidoglycan sacculus resulting from cell wall $_D$-alanylation may also sterically hinder AMP access to the cell membrane and contribute to AMP resistance (80). As a consequence, the cell wall of a GBS mutant lacking *dltA* was less compact and more permeable to AMPs than the WT parent strain (80). However, additional research is required to ascertain whether or not this mechanism applies to other Gram-positive species.

Compared to WT strains, *S. aureus dltA* null mutants and *dltA*, *dltB*, and *dltD* mutants of *Staphylococcus xylosusare* deficient in $_D$-alanine esters of LTAs are hypersensitive to

human α-defensins and cathelicidin due to an increase in negative surface charge and enhanced AMP binding (66, 72, 75). Furthermore, the overexpression of *dlt* in WT *S. aureus* enhances AMP resistance by increasing the cell surface positive charge (72). An *S. aureus dltA* mutant has reduced adherence to artificial surfaces, diminished biofilm formation, and reduced virulence in murine infection models (75, 81, 82). Several Gram-positive human pathogens have *dlt* operons, including GAS (71), GBS (66), *Streptococcus pneumoniae* (68), *Enterococcus faecalis* (67), *Listeria monocytogenes* (83), and *B. subtilis*. Inactivation of *dltA* in these and other Gram-positive species enhances sensitivity to human α-defensins and cathelicidin LL-37 (66, 71, 72, 83). Correspondingly, LTA $_{D}$-alanylation is required for full virulence in mouse models of GAS (71), *L. monocytogenes* (83), and GBS infection (66). $_{D}$-alanylation promotes GAS neutrophil intracellular survival as well as *L. monocytogenes in vivo* whole blood survival and *in vitro* adherence to macrophages, hepatocytes and epithelial cells (83). In *Lactobacillus*, TA $_{D}$-alanylation plays an important role in establishing gastrointestinal tract colonization (84).

Aminoacylation with $_{L}$-Lysine or $_{L}$-Alanine—The multiple peptide resistance factor MprF (also known as LysS), encoded by the *mprF* gene, is highly conserved ~97 kDa integral membrane protein found in both Gram-positive and Gram-negative bacteria. MprF possesses a conserved C- terminal hydrophilic cytoplasmic domain and a large N-terminal flippase domain (85) that reduces the net negative surface charge of Gram-positive bacteria by incorporating $_{L}$-lysine or $_{L}$-alanine into cell wall PG (82, 85, 86). MprF is a lysine-substituted phophatidylglcerol (L-PG) synthase that alters surface charge through the formation of a positively charged membrane phospholipid (82, 87) (Fig. 1A). Both the N- and C-terminal domains of MprF are necessary for AMP resistance in *S. aureus* (85) and *mprF* gene expression is controlled by the ApsRSX regulator (88) A *S. aureus* mutant strain lacking *mprF* has an increase in negative surface charge compared to WT, is more sensitive to killing by a broad range of bacterial and mammalian AMPs, including neutrophil defensins (82), and is less virulent in mouse infection models (75, 81, 82).

Aminoacylation of PG by MprF homologues increases AMP resistance in multiple bacterial species, including *Clostridium perfringens*, *E. faecalis*, *P. aeruginosa*, *Mycobacterium tuberculosis*, *Bacillus anthracis*, *B. subtilis*, *Enterococcus faecium*, and *L. monocytogenes* (89). *C. perfringens*, a Gram-positive spore-forming bacterium and common cause of foodborne illness, expresses two *mprF* genes designated *mprF1* and *mprF2*, which encode for alanyl phosphatidylglycerol synthase (A-PGS) and lysylphosphatidylglycerol synthase (L-PGS), respectively (86, 90). In *M. tuberculosis*, the addition of positively charged amino acid $_{L}$-lysine to PG is encoded by the *lysX* gene encoding for MprF homolog LysX, and is essential for resistance to cationic antibiotics and AMPs (91, 92). Lysinylation of PG has also been described for *L. monocytogenes* (93), *P. aeruginosa* (94), and *B. anthracis* (95).

Additional cell wall modifications in Gram-positive bacteria have also been reported to influence AMP resistance. In GBS, the *ponA* gene encodes for penicillin-binding protein 1a (PBP1a) and promotes resistance to human cathelicidin and defensins (96). The *pgm* gene, encoding a phosphoglucomutase, contributes to AMP resistance in porcine pathogen *Bordetella bronchiseptica* and the fish pathogen *Streptococcus iniae* (97, 98). In *Mycobacterium marinum*, mutation of the *kasB* gene, encoding beta-ketoacyl-acyl carrier

protein synthase B (KasB) reduces growth in human macrophages and bacterial survival in the presence of human defensins (99).

Modification of LPS with $_{L}$-Ara4N or pEtN—The outer membrane of Gram-negative bacteria is composed of lipid A, an anionic dimer of glucosamine linked to fatty acid chains and flanked by polar phosphate groups synthesized on the cytoplasmic surface of the inner membrane by highly conserved enzymes. The lipid A moiety has an attached core polysaccharide and species specific side-chain "O" polysaccharides (62). Modification of this complex, known as LPS, with amine substituents $_{L}$-Ara4N or pEtN reduces the net negative surface charge and AMP affinity, thereby promoting AMP resistance in Gram-negative bacteria such as *Salmonella* spp., important human pathogens and the causative agents of enteric/typhoid fever (Fig. 1A). In *S.* Typhimurium, two-component regulatory system PmrAB plays an important role in sensing extracellular cationic AMPs *in vivo*, and coordinates the expression of *pmrC* to decorate lipid A with ethanolamine, and *pmrEHFIJKLM* for the attachment of positively charged of $_{L}$-Ara4N to the 4'-phosphate group of the lipid A backbone, which together reduce the net negative charge of lipid A and enhance resistance to cationic AMPs (100, 101). All genes except for *pmrM* are required for the addition of $_{L}$-Ara4N and increased resistance to cationic AMPs in *S.* Typhimurium (100). *S.* Typhimurium lacking the LPS modifying enzyme PmrA are more sensitive to AMPs and have reduced virulence in a murine model of enteric infection (100, 102). $_{L}$-Ara4N modification of LPS enhances AMP resistance of several Gram-negative species including *Proteus mirabilis*, responsible for urinary tract infections (103); *Yersinia pseudotubercolosis*, a causative agent of enterocolitis (104); *K. pneumoniae*, a human lung pathogen (105); and *P. aeruginosa* (106), associated with chronic airway infections in cystic fibrosis patients (107).

In the Gram-negative pathogen *H. pylori*, an etiologic agent of peptic ulcers and increased gastrointestinal cancer risk, the addition of pEtN to dephosphorylated lipid A of LPS increases AMP resistance and reduces TLR4–mediated activation of the innate immune system (108, 109). Mutation of the *H. pylori lpxEHP* genes disrupted direct attachment of pEtN to the disaccharide backbone of lipid A, increased net negative charge of LPS and concomitantly reduced the minimum inhibitory concentration (MIC) of polymyxin B, a bacterial-derived AMP, by 25-fold compared to WT (108). In *Neisseria gonorrhoeae*, the *lptA* gene catalyzes addition of pEtN to lipid A and is necessary for polymyxin B resistance and survival in humans (110). Similarly, mutagenesis of *lptA* in *Neisseria meningitidis* decreased resistance to polymyxin B, protegrin-1 (PG1) and LL-37 (111). Deletion of the *lpxA* gene encoding an enzyme in the lipid A biosynthesis pathway of *N. meningitidis*, abolishes lipooligosaccharide (LOS) production and increases sensitivity to cationic AMPs (112, 113). Similarly, mutation of *waaF*, *cstII*, *galT* or *lgtF* genes in *Campylobacter jejuni* results in LOS truncation and hypersensitivity to AMPs including polymyxin B, human α-defensin-5 (HD-5), and the murine HD-5 homologue Crp4 (114, 115).

Acylation and Phosphorylcholination of LPS—The *pagP* gene encoding acetyltransferase PagP in the outer membrane of *S.* Typhimurium acylates lipid A and increases AMP (C18G, pGLa, and PG1) resistance by reducing outer membrane

permeability (63, 116) (Fig. 1A). Inactivation of the *pagP* homologue *rcp* in respiratory tract pathogen *L. pneumophila* reduces growth rate, AMP resistance, intracellular survival, and mouse lung colonization (117). LOS acylation by the *H. influenzae htrB* gene product is required for resistance to human AMP β-defensin 2 (HBD-2) (118). Addition of phosphorylcholine (ChoP) to the oligosaccharide portion of LPS promotes *H. influenzae* resistance to human cathelicidin LL-37 (119), conceivably through the cell surface exposure of the positively charged quaternary amine on choline to promote electrostatic repulsion (Fig. 1A). Inactivation of the *lpxM* gene in *K. pneumoniae*, which encodes an enzyme necessary for secondary acylation of immature lipid A, increases sensitivity to α-helical cationic AMPs through enhanced outer membrane permeability (120). In pathogenic *Vibrio cholerae* strain El Tor, the *msbB* gene is required for full acylation of the lipid A moiety and resistance to cationic AMPs (121).

Trapping of AMPs by Surface Molecules

Proteins and polysaccharides associated with the bacterial surface or secreted into the extracellular milieu may directly bind AMPs (Fig. 1B), thereby blocking access to the cytoplasmic membrane target of action and the formation of lytic pores. Another indirect AMP neutralization strategy employed by bacterial pathogens involves the release of the bound AMP from the bacterial surface (Table 2).

Surface-associated Proteins, Secreted Proteins and Polysaccharides—

Plasminogen is the inactive form of plasmin, a host serine protease involved in the degradation of blood clots and tissue remodeling. *S. aureus* secretes a plasminogen activating protein known as staphylokinase (SK). The accumulation of active plasmin activity on the *S. aureus* cell surface promotes host tissue invasion and dissemination to normally sterile sties (122). SK binds and inactivates mCRAMP and α-defensins released from human neutrophils including HNP 1-3 (122, 123) (Fig. 1B), reducing AMP activity against *S. aureus* by more than 80%. Further, *S. aureus* strains expressing SK are more resistant to killing by α-defensins in a mouse model of arthritis, and the addition of purified SK to SK-deficient strains enhanced survival in the presence of α-defensin *in vitro* (123). The secreted hydrophilic GAS protein streptococcal inhibitor of complement (SIC) binds and inactivates human LL-37, α-defensin and lysozyme to promote bacterial survival (Fig. 1B) (124-126). A *sic* knockout mutant in the highly invasive M1T1 GAS genetic background was more sensitive to killing by AMPs, and shows diminished virulence in animal infection models (124, 125).

The M protein of GAS, encoded by the *emm* gene, is a major cell wall-anchored coiled-coil protein required for resistance to opsonophagocytosis, adherence to host cells, and full virulence in animal models of GAS infection (127). The C-terminal region of M protein is highly conserved and contains the canonical LPXTG well wall anchor motif. GAS is classified into *emm* types according to the nucleotide sequence of the hypervariable N-terminal region. Currently, there are more than 200 known GAS serotypes and the M1 GAS serotype is the most frequently isolated serotype from invasive GAS infections worldwide (128, 129). Mutation of the *emm1* gene, encoding M1 protein, significantly increased the sensitivity to LL-37 or mCRAMP compared to WT (130), while the heterologous expression

of M1 protein in serotype M49 GAS or *Lactococcus lactis* enhanced LL-37 resistance. The trapping of LL-37 through the hypervariable extracellular N-terminal domain of M protein impedes LL-37 access to the cell membrane and promotes bacterial survival in LL-37-containing neutrophil extracellular traps (NETs) (Fig. 1B) (130). In GBS, surface-associated penicillin-binding protein-1a and the PilB surface pilus protein promotes adherence to host cells and resistance to cathelicidin AMPs through surface sequestration of LL-37 and mCRAMP *in vitro* (131, 132). Inactivation of *pilB* in GBS also reduces virulence in a mouse infection model (132).

Serological classification of streptococci in groups is based upon expression of unique carbohydrate antigens in the bacterial cell wall (133) known to play a structural role in cell wall biogenesis (134). Approximately 50% of the GAS cell wall by weight is made up of a single polysaccharide molecule termed the group A carbohydrate (GAC) antigen. All strains of GAS express GAC, composed of a polyrhamnose core with an immunodominant *N*-acetylglucosamine (GlcNAc) side chain (134). Inactivation of the *gacI* gene, encoding for a glycosyltransferase, abolished expression of the GlcNAc side chain in serotype M1 GAS. The *gacI* mutant was more susceptible to killing within NETs and to human cathelicidin LL-37, a component of neutrophil specific granules important for intracellular killing and deployed within NETs (135). Similarly, the *gacI* mutant had reduced growth in human serum and was hypersensitive to killing by the antimicrobial releasate from thrombin-activated human platelets. Loss of the GlcNAc epitope on GAC attenuated GAS virulence in a rabbit model of pulmonary infection, and a mouse model of systemic infection (135). In studies with purified WT and mutant GAC, the GlcNAc side chain was shown to impede LL-37 interaction with the underlying polyrhamnose core (135).

The active shedding of negatively charged surface exposed proteoglycans on host epithelial cells by proteases from bacterial pathogens is another resistance mechanism to trap and inactivate AMPs in tissues (Fig. 1B). Proteases secreted by GAS, *E. faecalis* and *P. aeruginosa* degrade decorin and release dermatan sulfate, which can bind and inactivate human α defensin HNP-1 (136). Syndecan-1, a proteoglycan derived from the degradation of heparan sulfate, is released from the host cell surface by *P. aeruginosa* virulence factor LasA to bind and impede AMP function (137). *S. epidermidis* synthesizes polysaccharide intercellular adhesin (PIA), a positively charged extracellular matrix polymer encoded by the *ica* gene locus (*icaADBC*) and the *cap* gene (138), to enhance electrostatic repulsion and resistance to cationic AMPs LL-37 and human β-defensin-3 (HBD-3) (139-141).

Capsular Polysaccharides—Several bacterial pathogens express surface capsules composed of high molecular mass polysaccharides that promote *in vivo* survival and trap cationic AMPs to impede interactions with the microbial cell surface (Fig. 1B). The hyaluronan capsule of GAS promotes survival in NETs through enhanced resistance to LL-37 (142). In *K. pneumoniae*, the *cps* capsule biosynthesis operon is transcriptionally upregulated in the presence of AMPs to enhance resistance to polymyxin B, protamine sulfate, defensin-1, β-defensin-1, and lactoferrin (143). The capsule of *K. pneumoniae* prevents engagement of TLR 2 and 4 and subsequent activation of the nuclear factor-κB (NF-κb) and mitogen-activated protein kinases (MAPK) pathways to inhibit the expression of human β-defensins (144). Administration of capsular polysaccharide extracts from *S.*

pneumoniae serotype 3 and *P. aeruginosa* enhanced the resistance of non-encapsulated *K. pneumoniae* to α-defensin HNP-1 and polymyxin B, suggesting that the release of capsule from the bacterial surface promotes the trapping of AMPs to prevent access to the site of action (145). Further, polymyxin B and HNP-1 also stimulate the release of capsule from the *S. pneumoniae* cell surface to sequester AMPs and increase AMP resistance (145). Studies with encapsulated WT and nonencapsulated serotype B mutant *N. meningitidis* demonstrate that capsule promotes resistance to protegrins, α- and β-defensins, polymyxin B, and cathelicidins LL-37 and mCRAMP (146). Moreover, the release of capsule from the surface of *N. meningitidis* is reported to promote resistance to LL-37 (113), and sub-lethal concentrations of AMP induce capsule biosynthesis (113, 146). Other bacterial species shield AMP targets with surface polymers. For example, LOS expression in *C. jejuni* increases LL-37, α-defensins and polymyxin B resistance (114). *P. aeruginosa* biofilms produce alginate polysaccharide, a polymer of β-$_D$-manuronate and α-$_L$-guluronate, to sequester and induce AMP conformational changes and peptide aggregation to prevent AMP access to the cell membrane (147).

Efflux Systems for AMP Resistance

Well-studied for their prominent role in resistance to pharmaceutical antibiotics, certain adenosine triphosphate (ATP)-binding cassette (ABC) driven efflux pumps are used by human bacterial pathogens to resist AMPs through the extrusion of AMPs from the cell membrane site of action to the extracellular environment (148) (Fig. 1C). Three major classes of ABC transporter systems play a role in AMP resistance, including 1) three-component ABC-transporters, 2) two-component ABC-transporters, and 3) single protein multidrug-resistance transporters (149). Several three-component ABC transporters implicated in AMP resistance have been described in Gram-positive species, including NisFEG (*L. lactis*) (150), SpaFEG (*B. subtilis*) (151), and CprABC (*Clostridium difficile*) (152, 153) (Table 2). Common two-component systems involved in AMP resistance include the BceAB transporter system identified in *B. subtilis* (154), *S. aureus* (155), *L. lactis* (156), *S. pneumoniae* (157) and *L. monocytogenes* (158), and the BcrAB(C) transporter identified in some species of *Bacillus* (159), *Enterococcus* (160), *Clostridium* (161) and *Streptococcus* (162). The energy-driven efflux pumps RosA/RosB and AcrAB are required for polymyxin B resistance in *Y. enterocolitica* and *K. pneumoniae*, respectively (163, 164). In *Y. enterocolitica*, RosA and RosB upregulate the *ros* locus and are necessary and sufficient for resistance to cationic AMPs. In *K. pneumoniae*, AacrAB also enhances resistance to α- and β-defensins (164). The MefE/Mel efflux pump contributes to LL-37 resistance in *S. pneumoniae* (165), while the TrkA and SapG potassium transport proteins in *Vibrio vulnificus* and *S.* Typhimurium, respectively, are essential for cationic AMP resistance (166, 167).

The energy-dependent MtrCDE efflux pump is a member of the resistance-nodulation-division (RND) efflux family. In the pathogens *N. gonorrhoeae* and *N. meningitidis*, MtrCDE is involved in actively transporting AMPs out of the bacterial cytoplasm and periplasmic space to promote resistance to LL-37, mCRAMP, PC-8, TP-1 and PG1 (61, 111, 168). In addition, the Mtr efflux pump increases resistance to β-lactam and macrolide antibiotics and *in vivo* resistance to innate immune clearance (61, 168, 169). MtrCDE is

necessary for *N. gonorrhoeae* colonization in a mouse model of genital tract infection (170), and inactivation of *mtrC* in *Haemophilus ducreyi* induces hypersensitivity to β-defensins and human LL-37 (171). The *sapABCDF* operon encoding ABC importer Sap ('sensitive to antimicrobial peptides') in *S.* Typhimurium enhances resistance to protamine, bee-derived AMP melittin, and crude extracts from human neutrophil granule extracts (167, 172, 173). The Sap transporter also contributes to AMP resistance in other Gram-negative species, including *H. influenzae* (172) and *H. ducreyi* (174). Deletion of the *S.* Typhimurium *yejF* gene from the *yejABEF* operon encoding an ABC-type peptide import system, reduced resistance to polymyxin B, melittin, protamine, and human β-defensins 1 and 2 (175). In *S. aureus*, single protein efflux pump QacA, encoded on naturally occurring plasmid pSK1, belongs to the major facilitator superfamily of transport proteins and uses proton motive force to extrude substrates (176). QacA promotes resistance to rabbit platelet AMP and host-derived thrombin-induced platelet microbicidal protein (tPMP-1) (177), and may also induce secondary changes in membrane fluidity to promote AMP resistance (178). Increased resistance to tPMP-1 in *S. aureus* is correlated with *in vivo* survival in animal infection models, and endocarditis in humans (177, 179).

Inactivation of AMPs by Proteolytic Degradation

AMPs are relatively resistant to proteolytic degradation by surface-associated or secreted proteases produced by bacterial pathogens (180). However, some bacterial proteases with broad substrate specificity promote disease pathogenesis by efficiently cleaving and inactivating AMPs (Fig. 1D). The human AMP LL-37 is cleaved into nonfunctional breakdown products by proteases expressed by several human pathogens including *E. faecalis* (metallopeptidase gelatinase) (181), GAS (broad-spectrum cysteine protease SpeB) (182), *S. aureus* (aureolysin) (183), and *P. mirabilis* (50 kDa metalloprotease) (184). Aureolysin inactivates LL-37 by cleaving the C-terminal peptide bonds between the Arg_{19}-Ile_{20}, Arg_{23}-Ile_{24} and Leu_{31}-Val_{32} (183), and promotes survival within the LL-37 rich environment of macrophage phagolysosomes (185). The GAS protease inhibitor α2-macroglobulin (α2M) binds broad-spectrum cysteine protease SpeB to the cell surface with the help of surface-associated G-related α2M-binding (GRAB) protein to facilitate LL-37 cleavage and bacterial survival (186, 187). The metalloprotease ZapA, a major virulence factor of *P. mirabilis* that degrades antibodies, extracellular matrix molecules, and complement components C1q and C3, also contributes to AMP resistance by cleaving human β-defensin 1, LL-37 and PG1 (184). The elastase of *P. aeruginosa* completely degrades and inactivates LL-37, promoting survival in an *ex vivo* wound fluid model (181). The *S.* Typhimurium *pgtE* gene that encodes for outer membrane protease PgtE, enhances resistance to LL-37 and C18G, an α-helical cationic AMP (188). Plasminogen-activating streptokinase secreted by GAS results in the accumulation of cell surface plasmin activity capable of degrading LL-37 (189). In *Burkholderia cenocepacia*, ZmpA and ZmpB zinc-dependent metalloproteases cleave and inactivate AMPs LL-37 and β-defensin 1, respectively (190). High-level expression of outer membrane protease OmpT of enterohemorrhagic *E. coli* (EHEC) promotes AMP resistance through the efficient degradation of LL-37 at dibasic sites (191). Proteases secreted by other pathogens also efficiently cleave and inactivate AMPs, including *B. anthracis* (LL-37), *Porphyromonas*

gingivalis (α- and β-defensins, cecropin B), and *Prevotella* spp. (brevinin) (192-196) (Table 2).

Regulatory Networks and AMP Resistance

Bacterial pathogens use two-component regulatory systems to modulate gene expression in response to extracellular metal ion concentrations, metabolic requirements, growth phase, or to subvert the host innate immune response mounted by neutrophils or macrophages within host tissue, resulting in the up- or down-regulation of genes necessary for survival and disease progression. Several pathogens achieve maximal resistance to AMPs through the coordinated transcriptional up-regulation of AMP resistance factors (Fig. 1E). PhoPQ is a well-studied two-component system in *S.* Typhimurium that responds to changes in magnesium ion (Mg^{2+}) concentration, pH and the presence of cationic AMPs (20, 197) (Table 2). Sensor kinase PhoP directly or indirectly coordinates the expression of >100 genes in *S.* Typhimurium encoding for proteins involved in Mg^{2+} transport (MgtA and MgtCB), transcriptional regulators important for intracellular macrophage survival (SlyA), oxidative stress resistance (RpoS), LPS modification by amino arabinose (PmrAB), and lipid A acylation (PagP) to reduce the fluidity and permeability of the bacterial membrane and enhance AMP resistance (20, 188, 198). Consequently, PhoPQ plays a role in modifying LPS surface charge (63), in enhancing macrophage resistance through the up-regulation of the AMP-degrading outer membrane protease PgtE (188, 199-201), and is required for full virulence in a mouse model of gastrointestinal infection (167). Additional *S.* Typhimurium transcriptional factors associated with resistance to bacterially derived AMP polymyxin B include *virK*, *somA*, and *rcsC* (202). PhoPQ homologs have been identified in other Gram-negative pathogens, including *Yersinia pestis*, *Shigella flexneri*, and *P. aeruginosa* (203). Mutant strains of *Y. pestis* deficient in PhoPQ are more sensitive to AMPs and neutrophil intracellular killing (204). In *P. aeruginosa*, the presence of AMPs or divalent cations activates the PhoPQ and PmrAB systems to enhance resistance to cationic AMPs such as LL-37 and polymyxin B (205-207). The two-component system PmrAB in *P. aeruginosa* co-ordinates the incorporation of positively charged $_{L}$-Ara4N subunits into LPS and promotes AMP resistance through electrostatic repulsion (106, 206).

Upon encountering bacteria at the site of infection, NETs are released to help trap and kill the bacteria. NETs are a composed of DNA backbone and antimicrobial effectors such as histones, granule proteases and AMPs (in particular cathelicidin) that promote microbe killing (208, 209). Degradation of the DNA scaffold by secreted bacterial DNAses promotes NET escape and survival for several bacterial pathogens including GAS (210-212), *S. pneumoniae* (213), GBS (214) and *S. aureus* (215). Subinhibitory concentrations of exogenous DNA promote *P. aeruginosa* AMP resistance through the chelation of divalent cations and the resultant upregulation of AMP resistance genes (216). In *S.* Typhimurium, extracellular DNA also induces *pmr* expression and AMP resistance (217).

The $_{D}$-alanylation of techoic acid by the *dlt* operon is regulated by the *agr* locus in *S. aureus* and promotes AMP resistance (Dunman et al., 2001). Exposure of *S. aureus* to AMPs activates the VraSR and VraDE operons involved in resistance to AMPs and cell wall-targeting antibiotics such as bacitracin (28). Human β-defensin (HBD-3) triggers the

upregulation of the cell wall stress response pathway in *S. aureus* to counteract HBD-3-induced perturbation of peptidoglycan synthesis (13). Exposure of *S. aureus* to sub-lethal concentrations of magainin 2 and gramicidin D promotes to resistance to these AMPs through the enhancement of membrane rigidity (218). Changes in membrane fluidity induced by incorporation of longer chain unsaturated fatty acids into the lipid bilayer (resulting in increased membrane fluidity), or carotenoid staphyloxanthin pigment (resulting in increased membrane rigidity), promotes *S. aureus* resistance to platelet-derived AMPs (tPMPs), or polymyxin B and human neutrophil defensin 1, respectively (219, 220). While the precise resistance mechanism has yet to be determined, a significant increase or reduction in membrane fluidity may hinder AMP insertion into the cellular membrane (89, 221). In *L. monocytogenes*, an increase in the concentration of membrane saturated fatty acids and phophatidylethanolamine, and a decrease in phophatidylglycerol concentration, reduces the fluidity of the cell membrane to promote nisin resistance (222, 223). PrfA, a temperature-regulated transcription factor in *L. monocytogenes*, contributes to defensin resistance (224).

Modulation of Host AMP Production by Bacterial Pathogens

While low levels of AMPs are produced by epithelial and host immune cells at baseline, AMP expression is typically dramatically upregulated in response to bacterial infection. Some bacterial pathogens resist AMP-mediated innate immune clearance by interfering with, or suppressing, host AMP expression levels (Fig. 1F). *Shigella* spp. are Gram-negative rods capable of causing life-threatening invasive human infections such as bacillary dysentery. *Shigella dysenteriae* and *S. flexneri* downregulate the expression of LL-37 and β-defensin-1 in intestinal epithelial cells during early infection through a mechanism dependent on transcriptional factor MxiE and the type III secretion system to promote bacterial survival, colonization and invasion of the gastrointestinal tract (225, 226) (Table 2). *P. aeruginosa*, a human pathogen commonly isolated from the lungs of cystic fibrosis patients, induces the expression of host cysteine proteases cathepsins B, L and S to cleave and inactivate β-defensins 2 and 3 and thwart AMP-mediated clearance of the bacteria in airway fluid (227). Enterotoxigenic *E. coli* (ETEC) and *V. cholerae* exotoxins reportedly repress the expression of host cell HBD-1 and LL-37 (228), while *N. gonorrhoeae* downregulates the expression of AMP genes (229). *Burkholderia* spp. are human pathogens associated with opportunistic infections in cystic fibrosis patients and chronic granulomatous disease (230). The high level AMP resistance exhibited by this Genus has been attributed to the constitutive incorporation of $_{L}$-Ara4N into the LPS molecule (230, 231). Alternative sigma factor RpoE coordinates *Burkholderia* gene expression under stress conditions and contributes to AMP resistance in a temperature-dependent manner (230, 232).

Concluding Remarks and Future Directions

AMPs are present in most organisms and are an ancient and diverse group of naturally occurring anti-infective molecules that play an integral part in the host innate immune defense against bacterial infection. Bacterial AMP resistance mechanisms have evolved as a result of selection pressures from direct competition among species (bacteriocins) and during host-pathogen interactions (innate defense AMPs). Human bacterial pathogens have

evolved a broad diversity of intrinsic or inducible AMP-defense mechanisms to promote survival, colonization, and subsequent dissemination to normally sterile sites within the body to cause life-threatening invasive syndromes. Bacterial pathogens with intrinsic high-level resistance to AMPs, such as *S. aureus* and *Salmonella* spp. can bypass normally effective mucosal defenses and are consequently among the leading causes of causes of deep tissue and systemic infections. AMP resistance is mediated by a variety of different molecular mechanisms including net cell surface charge alteration, efflux, restricting AMP access to their targets, and proteolytic cleavage of AMPs. Bacterial mutants sensitive to AMPs in *in vitro* assays are attenuated for virulence in systemic animal infection models. An improved comprehension of AMP modes of action, resistance mechanisms and host pathogen interactions may inspire the development of alternative antibacterial therapeutics that target the cell wall, efflux pumps, or AMP-inactivating proteases, ultimately enhancing bacterial sensitivity to the AMPs of the host innate immune system. Understanding the interaction between conventional antibiotics and endogenous AMPs can also lead to improved therapeutic strategies for drug-resistant pathogens. An action of beta-lactam antibiotics to sensitize methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* spp. (VRE) to killing by human cathelicidin LL-37 and cationic peptide antibiotic daptomycin has shown promise in synergy studies and small clinical series in patients with previously recalcitrant infections (233, 234).

The emergence of antibiotic resistant microbes through the excessive and inappropriate use of conventional antibiotics is a critical public health threat responsible for high morbidity and rates and significant socioeconomic costs worldwide. Moreover, the antibiotic development pipelines of the major pharmaceutical companies have steadily declined over the past 20 years. Consequently, there is considerable interest in alternative therapeutic approaches to facilitate the fight against multidrug-resistant pathogens, including the development of novel broad-spectrum AMPs against bacteria, fungi, protozoa and enveloped viruses (30, 235). Importantly, the AMP mechanism of action is very rapid at concentrations close to the MIC, in comparison to conventional antibiotics (236). In recent years, intensive research and has led to the establishment of several bioinformatics tools and databases (e.g. APD2, CAMP, iAMP-2L) to identify and isolate new AMP classes and to elucidate their structure, function and biological activity (237). However, prolonged *in vitro* exposure of bacteria to sub-lethal AMP concentrations (238), and pre-clinical trials with naturally occurring cationic AMPs have detected resistant strains, indicating that optimization of AMP composition and structures are required to enhance stability and efficacy (237). Cross-resistance to AMPs with disparate modes of action has also been reported. For example, *S. aureus* is resistant to pexiganan and cross-resistant to HNP-1 (239). *S. aureus* isolates resistant to daptomycin, a cyclic lipopeptide antibiotic that associates with Ca^{2+} to form a cationic complex (240), are also more resistant host defense AMPs with diverse mechanisms of action, including HNP-1, polymyxin B, and tPMPs (241). Human pathogens resistant to nisin, an AMP used as a food preservative (*L. monocytogenes*, *Streptococcus bovis*) (242, 243), and colistin, also known as polymyxin E (*Acinetobacter baumannii*, *P. aeruginosa*, *Brevundimonas diminuta*, *Ochrobactrum anthropic*, *K. pneumoniae*) (244, 245) have recently been reported.

The transfer of broad-spectrum resistance mechanisms between bacteria and the development of resistance against our own host defense peptides remain valid concerns moving forward with the development of AMPs for clinical use (246, 247). Systemic toxicity and decreased blood and/or serum activity of natural peptides have significantly hampered clinical AMP development and provided the impetus for *de novo* designed peptide sequences (1). To this end, multiple new classes of AMPs have been reported (e.g. mimetic peptides, hybrid peptides, peptide congeners, stabilized AMPs, peptide conjugates, immobilized peptides) with potential application in medicine, veterinary medicine, and agriculture (248). Rationally designed synthetic AMPs have recently been demonstrated to be active against antibiotic-resistant *A. baumannii* and *K. pneumoniae* (249). Synthetic peptides could also be designed to resist bacterial and host proteases through the incorporation of $_{D}$-amino acids (229). While pathogenic bacteria have successfully evolved AMP-resistance mechanisms, resistance to a broad range of AMPs has not yet occurred. Enhanced microbicidal activity of phagocytic cells and enhanced resistance to bacterial infection *in vivo* has been achieved by genetic or pharmacological augmentation of transcriptional regulator hypoxia-inducible factor (HIF) (250, 251), which regulates the expression of human and murine cathelicidin at the transcriptional level (250, 252). Combination therapy with AMPs and classical antibiotics that target more than one site of action, such as the inhibition of cell wall synthesis coupled with cell membrane disruption, may help to combat the increasing emergence of multidrug-resistant microbes associated with challenging and deadly microbial infections.

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Abbreviations

α2M	α2-macroglobulin
ABC	adenosine triphosphate-binding cassette
AMPs	antimicrobial peptides
A-PGS	alanyl phosphatidylglycerol synthase
$_{L}$-Ara4N	4-amino-4-deoxy-$_{L}$-arabinose
ATP	adenosine triphosphate
CAMP	cathelicidin antimicrobial peptide
ChoP	phosphorylcholine
Dcl	$_{D}$-alanyl carrier protein ligase

Dcp	D-alanine carrier protein
EHEC	enterohemorrhagic *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
GAC	group A carbohydrate
GAS	group A *Streptococcus*
GBS	group B *Streptococcus*
GlcNAc	*N*-acetylglucosamine
GRAB	protein G-related α2M-binding protein
HBD 1-6	human β-defensin 1-6
HD 5-6	human α-defensin 5-6
HIF	hypoxia-inducible factor
HNP 1-4	human neutrophil peptide 1-4
KasB	beta-ketoacyl-acyl carrier protein synthase B
LL-37	human cathelicidin
LOS	lipooligosaccharide
L-PG	lysine-substituted phophatidylglcerol
LPGS	lysylphosphatidylglycerol synthase
LPS	lipopolysaccharide
LTA	lipoteichoic acid
MAPK	mitogen-activated protein kinases
mCRAMP	murine cathelicidin-related antimicrobial peptide
MIC	minimum inhibitory concentration
MprF	membrane protein multipeptide resistance factor
MRSA	methicillin-resistant *S. aureus*
NETs	neutrophil extracellular traps
NF-κb	nuclear factor-κB
PBP1a	penicillin-binding protein 1a
pEtN	phosphoethanolamine
PG	phosphatidylglycerol
PG1	protegrin-1
PIA	polysaccharide intercellular adhesion
RND	resistance-nodulation-division efflux family

Sap	sensitive to antimicrobial peptides ABC importer
SIC	streptococcal inhibitor of complement
SK	staphylokinase
SpeB	streptococcal pyrogenic exotoxin B
TA	teichoic acid
TLR	toll-like receptor
tPMP-1	thrombin-induced platelet microbicidal protein
TP-1	tachyplesin-1
VRE	vancomycin-resistant *Enterococcus*
WT	wild-type
WTAs	wall teichoic acids

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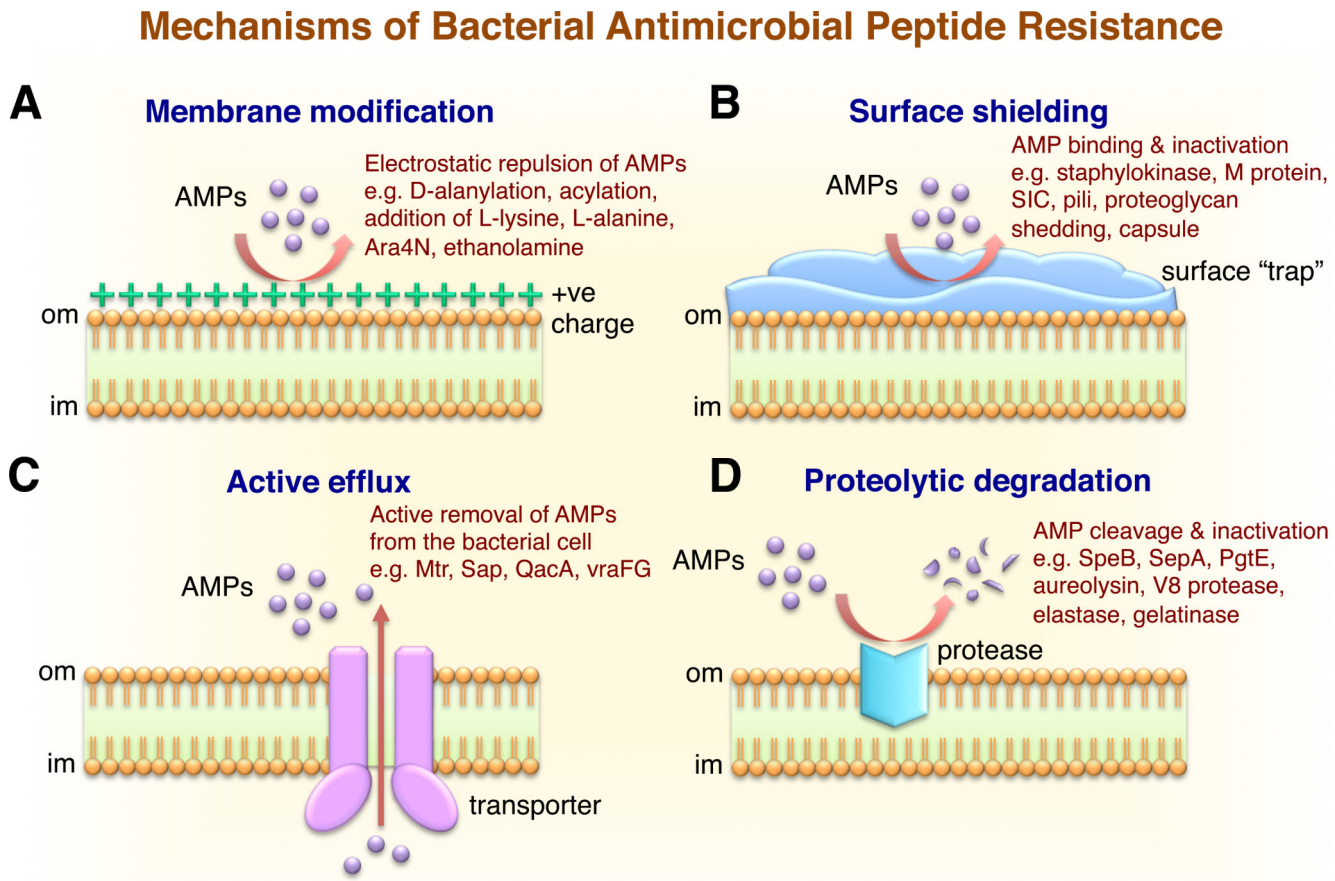

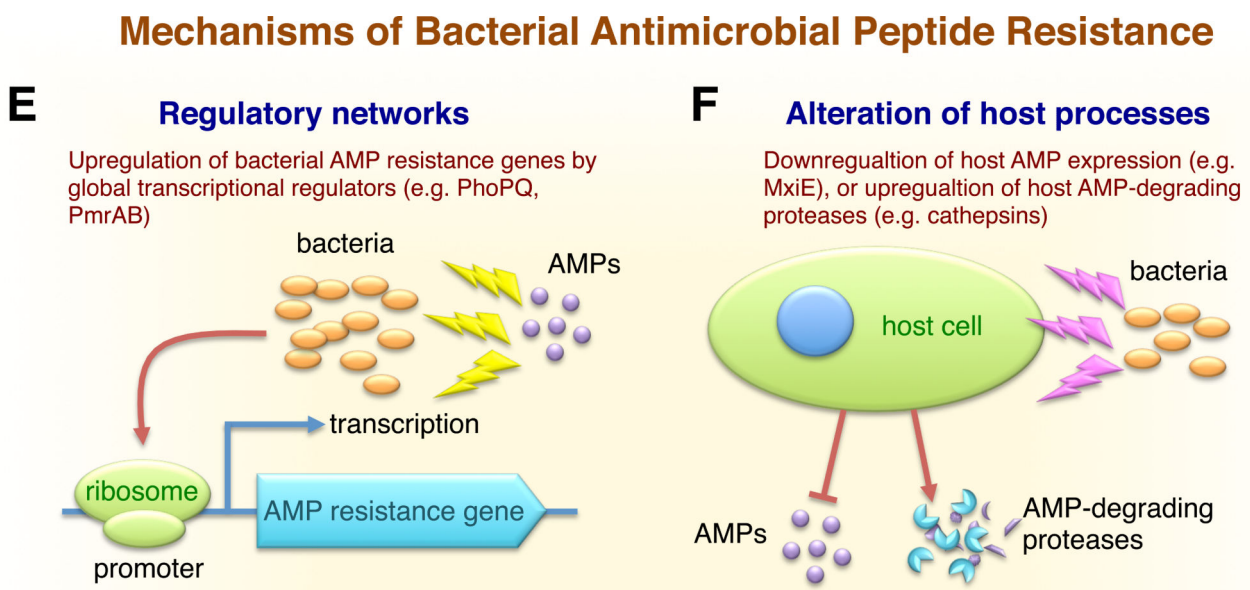

Figure 1.

Schematic representation of the multiple resistance mechanisms developed by bacteria to overcome host antimicrobial peptides. A) Modification of the bacterial outer membrane. Bacterial resistance to cationic antimicrobial peptides is mediated by alterations in surface charge. Gram-positive bacteria: $_{D}$-alanine modification of cell wall techoic acid (*dlt*), $_{L}$-lysine (*mprf*), or $_{L}$-alanine modification of phosphatidylglycerol (*mprf*). Gram-negative bacteria: aminoarabinose or acylation modifications of lipid A in LPS (*pmr*, *pagP*), or addition of ethanolamine to lipid A (*pmrC, lptA*). The increased positive charge on bacterial surface repels cationic AMPs. B) Shielding of the bacterial surface through the trapping and inactivation of AMPs in the extracellular milieu enhances resistance and pathogenicity. Surface-associated capsule traps AMP (e.g. *K. pneumoniae cps* operon), surface protein binds AMP (e.g. GAS M1 protein, GBS PilB pilus protein), secreted protein binds AMP (e.g. GAS SIC protein or *S. aureus* staphylokinase), or bacterial proteases release host proteoglycans to block AMP (e.g. *P. aeruginosa* LasA). C) Membrane efflux pumps function by translocating the AMP out of cell (e.g. *Neisseria* spp. Mtr, *S.* Typhimurium Sap, *S. aureus* QacA, and *Staphylococcus* spp. VraFG). D) Degradation and inactivation of AMPs by bacterial proteases (e.g. GAS SpeB protease, *S. epidermidis* SepA, *S.* Typhimurium PgtE, *S. aureus* aureolysin and V8 protease, *P. aeruginosa* elastase, and *E. faecalis* gelatinase). E) Bacterial exposure to AMPs upregulates the expression of AMP-

resistance genes through global gene regulatory networks (e.g. *S.* Typhimurium and *P. aeruginosa* PhoPQ and PmrAB). F) Alteration of host processes by bacteria, including the downregulation of host AMP production (e.g. *Shigella* spp. transcriptional factor MxiE), or the upregulation and activation of host AMP-degrading proteases (e.g. *P. aeruginosa*). Abbreviations: om, bacterial outer membrane; im, bacterial inner membrane.

Table 1

Human antimicrobial peptides and murine cathelicidin mCRAMP.

Class	Peptide	Gene	Species	Producing Cells	Reference(s)	Amino Acid Sequence*
α-defensins	HNP-1	*DEFA1*	Human	Azurophilic granules of neutrophil granulocytes	(14)	$AC_1YC_2RIPAC_3IAGERRYGTC_2IYQGRLWAFC_3C_1$
	HNP-2	*DEFA1*	Human			$C_1YC_2RIPAC_3IAGERRYGTC_2IYQGRLWAFC_3C_1$
	HNP-3	*DEFA3*	Human			$DC_1YC_2RIPAC_3IAGERRYGTC_2IYQGRLWAFC_3C_1$
	HNP-4	*DEFA4*	Human			$VC_1SC_2RLVFC_3RRTELRVGNC_2LIGGVSFTYC_3C_1TRV$
	HD-5	*DEFA5*	Human	Paneth cells in small intestine and female urogenital tract	(15, 16)	$ATC_1YC_2RTGRC_3ATRESLSGVC_2EISGRLYRLC_3C_1R$
	HD-6	*DEFA6*	Human			$AFTC_1HC_2RRSC_3YSTEYSYGTC_2TVMGINHRFC_3C_1L$
β-defensins	HBD-1	*DEFB1*	Human	Epithelial cells, monocytes, macrophages and dendritic cells	(11, 17)	$DHYNC_1VSSGGQC_2LYSAC_3PIFTKIQGTC_2YRGKAKC_1C_3K$
	HBD-2	*DEFB4*	Human			$GIGDPVTC_1LKSGAIC_2HPVFC_3PRRYKQIGTC_2GLPGTKC_1C_3KKP$
	HBD-3	*DEFB103*	Human			$GIINTLQKYYC_1RVRGGRC_2AVLSC_3LPKEEQIGKC_2STRGRKC_1C_3RRKK$
	HBD-4	*DEFB104*	Human			$ELDRIC_1GYGTARC_2RKKC_3RSQEYRIGRC_2PNTYAC_1C_3LRK$
Cathelicidins	LL-37	*CAMP*	Human	Skin cells, gastrointestinal cells, neutrophils and myeloid bone marrow cells	(18)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
	mCRAMP	*Cnlp*	Murine			GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPEQ
Others	C18G	n/a	Synthetic	n/a	(253)	ALYKKLLKKLLKSAKKLG

Abbreviations: HNP-1, human neutrophil defensin 1; HNP-2, human neutrophil defensin 2; HNP-3, human neutrophil defensin-3; HNP-4, human neutrophil defensin-4; HD-5, human α-defensin-5; HD-6, human α-defensin-6; HBD-1, human β-defensin 1, HBD-2, human β-defensin 2; HBD-3, human β-defensin-3; HBD-4, human β-defensin-4; LL-37, human cathelicidin; mCRAMP, murine cathelicidin-related peptides; C18G, α-helical peptide derived from the carboxy terminus of platelet factor IV. Modified from Gruenheid and Moual 2012 (229).

*Numbers denote cysteine residues involved in disulfide bonds.

Table 2

Bacterial antimicrobial peptide resistance mechanisms.

AMP Resistance Mechanism	AMP Resistance Phenotype	Gene(s)	Target AMPs	Bacteria*	Reference(s)
Cell surface alterations	$_{D}$-alanylation of lipoteichoic acid and teichoic acid in bacterial cell wall	*dlt* operon *dltA*	Cecropin B, colistin, gallidermin, HNP1-3, indolicidin, mCRAMP, magainin II, nisin, polymyxin B, protegrin 1, 3 and 5, tachyplesin 1 and 3, daptomycin, vancomycin	*Staphylococcus aureus* *Listeria monocytogenes* Group B *Streptococcus* Group A *Streptococcus* *Streptococcus pneumoniae* *Streptococcus suis* *Enterococcus faecalis* *Bacillus anthracis* *Bacillus cereus* *Clostridium difficile*	(66-68, 71, 72, 83, 254-259)
	Addition of $_{L}$-lysine or $_{L}$-alanine to phosphatidylglycerol in cell membrane	*mprF* *lysC* *lysX* *PA0920*	Arenicin-1, CAP18, gallidermin, HBD-3, HNP1-3, LL-37, lugworm beta-sheet peptide, lysozyme, magainin II, melittin, nisin, NK-2, polymyxin B, protamine, protegrin 3 and 5, tachyplesin 1, vancomycin	*Staphylococcus aureus* *Bacillus anthracis* *Listeria monocytogenes* *Mycobacterium tuberculosis* *Pseudomonas aeruginosa*	(73, 81, 82, 85, 87, 91, 93-95)
	Synthesis and extension of lipooligosaccharide	*lpxA* *lgtF* *galT* *cstII*	Crp4, Fowl-1, HD-5, LL-37, polymyxin B	*Neisseria meningitidis* *Campylobacter jejuni*	(113-115)
	Addition of ethanolamine (pEtN) to lipid A	*waaF* *lpxE*$_{HP}$ *cj0256* *pmrC* *lptA*	LL-37, protegrin 1, polymyxin B	*Helicobacter pylori* *Campylobacter jejuni* *S. Typhimurium* *Neisseria gonorrhoeae* *Neisseria meningitidis*	(101, 108, 110, 111, 260)
	Addition of aminoarabinose to lipid A in LPS	*pmr* genes	C18G, HBD-2, polymyxin B, protegrin 1, synthetic protegrin analogs	*S. Typhimurium* *Proteus mirabilis* *Pseudomonas aeruginosa* *Klebsiella pneumoniae*	(100, 103, 105, 106)
	Acylation of lipid A in LPS	*pagP* *rcp* *htrB msbB* *lpxM*	C18G, colistin, CP28, HBD-2, LL-37, magainin II, mCRAMP, protegrin 1, PGLa, polymyxin B and E	*Salmonella spp.* *Legionella pneumophila* *Haemophilus influenzae* *Vibrio cholerae* *Klebsiella pneumoniae*	(63, 117, 118, 120, 121)
	Phosphorylcholine in LPS	*licD*	LL-37	*Haemophilus influenzae*	(119)
	Synthesis of polysaccharide capsule	*cps* *siaD* *sia operon* *ica genes* *cap* *hasABC*	HBD-1 and 3, HNP-1 and 2, lactoferrin, polymyxin B, protamine, mCRAMP, CRAMP-18, HNP-1 and 2, LL-37, protegrin 1, polymyxin B, β-defensin-1, 2 and 3	*Klebsiella pneumoniae* *Neisseria meningitidis* *Staphylococcus epidermidis* *Streptococcus pneumoniae* *Streptococcus pyogenes*	(113, 139-143, 146)
	PCN-binding protein PBP1a	*ponA*	HNP-1, LL-37, mCRAMP	Group B *Streptococcus*	(96)

AMP Resistance Mechanism	AMP Resistance Phenotype	Gene(s)	Target AMPs	Bacteria*	Reference(s)
	Mycolic acid synthesis	*kasB*	HNP-1, protamine, lysozyme	*Mycobacterium marinum*	(99)
	Production of carotenoids	*crtOPQMN*	HNP-1, thrombin-induced platelet microbicidal proteins (tPMPs), polymyxin B	*Staphylococcus aureus*	(261-263)
Binding and inactivation	Staphylokinase	*sak*	HNP-1 and 2	*Staphylococcus aureus*	(122, 123)
	M1 surface protein	*emm1*	LL-37	Group A *Streptococcus*	(130)
	SIC protein	*sic*	LL-37, α-defensins, lysozyme	Group A *Streptococcus*	(124, 125)
	Shedding of host proteoglycans	*lasA*	LL-37, HNP-1	*Pseudomonas aeruginosa* *Enterococcus faecalis* Group A *Streptococcus*	(136, 264)
	PilB	*pilB*	LL-37, mCRAMP, polymyxin B	Group B *Streptococcus*	(132)
	LciA	*lciA*	Lactococcin A	*Lactococcus lactis*	(265, 266)
	LanI lipoproteins	*lanI*	Lantibiotics	*Lactococcus lactis* *Bacillus subtilis*	(267-269)
Active efflux	ATP-dependent efflux system	*mtr* genes	LL-37, mCRAMP, PC-8, TP-1, protegrin-1 (PG1)	*Neisseria gonorrhoeae* *Neisseria meningitidis*	(111, 168, 170, 270)
	K^+-linked efflux pump	*sap*	Protamine	*S. Typhimurium*	(167)
	Plasmid-encoded efflux pump	*qacA*	Rabbit thrombin-induced platelet microbicidal protein (tPMP)	*Staphylococcus aureus*	(177)
	VraFG ABC transporter	*vraFG*	nisin, colistin, bacitracin, vancomycin, indolicidin, LL-37, hBD3	*Staphylococcus aureus* *Staphylococcus epidermidis*	(155, 271-274)
Proteolytic degradation	Elastase	*lasB*	LL-37	*Pseudomonas aeruginosa*	(181)
	Gelatinase	*gelE*	LL-37	*Enterococcus faecalis*	(181, 275)
	Metalloproteinase	*zapA* *aur* *degP*	LL-37, lactoferricin	*Proteus mirabilis* *Staphylococcus aureus* *Escherichia coli*	(181, 183, 184, 192)
	Cysteine protease	*speB* *ideS*	LL-37	Group A *Streptococcus*	(181, 182)
	Surface protease	*pgtE*	C18G	*S. Typhimurium*	(188)
	Gingipains (serine proteases)	*rgpA/B*	Cecropin B	*Porphyromonas gingivalis*	(193)
	Aureolysin	*aur*	LL-37	*Staphylococcus aureus*	(183, 276)
	V8 protease	*sspA*	LL-37	*Staphylococcus aureus*	(183)
	SepA protease	*sepA*	Dermcidin	*Staphylococcus epidermidis*	(277, 278)
Alteration of Host Processes	Downregulate AMP transcription	*mxiE*	LL-37, human beta-defensin-1, human beta-defensin HBD-3	*Shigella dysenteriae* *Shigella flexneri*	(225, 226, 279, 280)

AMP Resistance Mechanism	AMP Resistance Phenotype	Gene(s)	Target AMPs	Bacteria*	Reference(s)
				S. Typhimurium *Neisseria gonorrhoeae*	
	Stimulation of host cysteine proteases cathepsins	Unknown	HBD-2, HBD-3	*Pseudomonas aeruginosa*	(227, 281)
Regulatory Networks	Two-component regulator	*phoP/phoQ*	Defensins, protamine	*S. Typhimurium* *Pseudomonas aeruginosa*	(200, 205)
	Two-component regulator	*pmrA/pmrB*	Defensins, polymyxin B	*S. Typhimurium* *Pseudomonas aeruginosa*	(100, 206)
	Thermoregulated transcription factor	*prfA*	Defensins	*Listeria monocytogenes*	(224)

Abbreviations: C18G, α-helical peptide derived from the carboxy terminus of platelet factor IV; CAP18, cationic LPS-binding protein 18 from rabbit; CP28, α-helical synthetic cationic peptide based on the cecropin-mellitin hybrid peptide CEME; mCRAMP and CRAMP-18, murine cathelicidin-related peptides; Crp4, murine homologous to human α-defensin-5; Fowl-1, heterophil-derived cathelicidin homolog fowlicidin-1; HBD-1, human β-defensin 1, HBD-2, human β-defensin 2; HBD-3, human β-defensin-3; HD-5, human α-defensin-5; HNP-1, human neutrophil defensin 1; HNP-2, human neutrophil defensin 2; HNP-3, human neutrophil defensin-3; LL-37, human cathelicidin, C-terminal part of the human cationic antimicrobial protein (hCAP-18); NK-2, α-helical fragment of mammalian NK-lysin; PGLa, peptide starting with a glycine and ending with a leucine amide from magainin peptide family. Modified from Anaya-Lopez *et al.* (3).

*Not all bacteria are resistant to the CAMP indicated; please see reference for specific resistance profile.