Bacterial Resistance to Antimicrobial Peptides and Mechanisms (Postprint)

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Abstract

Antimicrobial peptides (AMPs) are a class of small peptides widely distributed in natural organisms, representing an essential component of the innate immune system. As alternatives to conventional antibiotics, AMPs have garnered significant attention; however, bacterial resistance to AMPs, like antibiotic resistance, cannot be ignored. This review primarily discusses bacterial resistance to AMPs and the underlying mechanisms from three perspectives: planktonic bacteria, biofilms, and signaling regulation.

Full Text

Preamble

Bacterial Resistance to Antimicrobial Peptides and Their Mechanisms

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Abstract: Antimicrobial peptides (AMPs) are a class of small peptide substances widely present in natural organisms, constituting essential components of the innate immune system. As potential alternatives to traditional antibiotics, AMPs have garnered widespread attention; however, bacterial resistance to AMPs, much like antibiotic resistance, cannot be ignored. This review summarizes bacterial resistance to AMPs and its mechanisms from three aspects: planktonic bacteria, biofilms, and signal regulation.

Keywords: bacteria; antimicrobial peptides; planktonic bacteria; biofilm; signal regulation; resistance; mechanism

Due to antibiotic misuse, many bacteria have evolved into highly drug-resistant strains. Antimicrobial peptides (AMPs), with their characteristic of being less prone to resistance development, have become the most promising alternative to traditional antibiotics. AMPs are mostly strongly cationic small molecular polypeptides that bind to the negatively charged bacterial cell membrane surface, causing membrane disruption and bacterial death. However, bacteria can develop resistance to AMPs through modifying bacterial surface molecules, secreting protective substances, upregulating or eliminating specific proteins, and forming biofilms [1].

Based on these resistance mechanisms, bacteria can be categorized into two types: planktonic bacteria arising from single-bacterial factors and biofilms arising from multi-bacterial factors. In recent years, research on AMP resistance has been increasing, and the extensive or improper use of AMPs will inevitably lead to the same serious consequences as antibiotics. Deepening research on AMP resistance will help design more effective AMPs and prevent the development of AMP resistance. This review will focus on elaborating bacterial resistance to AMPs and its mechanisms.

1 Resistance of Planktonic Bacteria to AMPs

The amphiphilic structural characteristic is key to the antibacterial function of AMPs, and the bacterial cell membrane surface is the primary target of AMPs (Figure 1 [Figure 1: see original paper]). Planktonic bacteria achieve resistance to AMPs through a series of changes in the cytoplasmic membrane and cell wall. The cytoplasmic membrane is a physical barrier protecting cellular contents, while the cell wall provides structural support ensuring the survival of most bacteria. Based on cell wall structural differences, bacteria can be divided into Gram-positive and Gram-negative bacteria. Gram-positive bacterial cell walls include a thick peptidoglycan layer and other carbohydrates, primarily composed of lipoteichoic acid (LTA) and wall teichoic acid; Gram-negative bacterial cell walls are more complex, including a thin peptidoglycan layer, lipoproteins, lipopolysaccharides, and an outer membrane [2]. Resistance to AMPs mostly originates from modifications of these structures.

1.1 Extracellular Polysaccharides and Proteins

Extracellular polysaccharides are polysaccharide polymers synthesized and secreted by Gram-positive/negative bacteria, mostly anionic. When covalently attached to the cell wall, they are called capsular polysaccharides. In Gram-positive bacteria, extracellular polysaccharides mostly attach to the peptidoglycan layer, where they can attract and bind AMPs, hindering their further function [4].

Gram-negative bacteria, such as *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*, tend to release extracellular polysaccharides outside the cell to trap AMPs. This secretory function helps reduce the num-

ber of AMPs on the bacterial surface. Additionally, studies have shown that inducing capsular polysaccharide variation in *K. pneumoniae* makes it more susceptible to AMPs and easier to bind with capsular polysaccharides [5-6].

Extracellular proteins enable bacterial resistance to AMPs by trapping and degrading them. Bacteria use various proteases such as metalloproteases, serine proteases, cysteine proteases, and aspartic proteases, which are either secreted or fixed on the bacterial surface to degrade AMPs and protect their target sites [7]. *Streptococcus dysgalactiae* subsp. *equisimilis* can synthesize proteins DrsG and DrsG2, which are homologous to streptococcal inhibitor of complement (SIC) and distantly related to SIC (DRS). These proteins can be secreted outside the cell and bind to LL-37 (an AMP), thereby inhibiting its activity [8].

1.2 Cell Membrane Modification

Bacterial cell membranes contain several phospholipids, such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and cardiolipin (CL) [9]. Regulating cell membrane components can not only change membrane surface charge but also alter membrane fluidity and rigidity. Thus, regulating bacterial cell membrane components is critical for bacterial survival and a key point for AMP resistance.

Cell membranes can be divided into two models: (1) Membranes containing only 10% CL, which can effectively prevent membrane translocation and pore formation under AMP action. Penicillin-resistant *Staphylococcus aureus* strains utilize adaptation factors present in the membrane, through multiple peptide resistance factor (mrpF), cardiolipin synthase (cls), phosphatidylglycerol synthase (pgsA), and other coordinated mechanisms to modify the membrane surface to present positive charge, generating resistance to AMPs [10]. (2) Membranes containing aminoacyl phospholipids [lysine (Lys)-PE, glutamine (Gln)-PE], which is a common way for pathogenic bacteria to modify lipid bilayers to resist AMPs [11].

Lipid modifications can be divided into two categories: (1) PG modification. In Gram-positive bacteria, the gene encodes a large amount of membrane protein mrpF. To increase lysine and alanine content, PG synthesizes lysyl-phosphatidylglycerol (LPG) and alanyl-phosphatidylglycerol (APG), while transferring these substances to the exterior [12]. Compared with susceptible strains, *S. aureus* strains with functional mrpF mutations and containing large amounts of LPG have been found to have higher resistance to AMPs and reduced surface charge [10]. (2) Changes in fatty acid composition. Reducing branched-chain fatty acid content may increase the rigidity of *Enterococcus faecalis* membranes and accompany increased positive charge content, thereby preventing AMP penetration [13]. Similarly, increasing the content of unsaturated, branched-chain, and hydrogenated fatty acids in *S. aureus* cell membranes can give the membrane higher fluidity, preventing AMP aggregation [14].

1.3.1 Gram-Negative Bacteria

On the surface of Gram-negative bacteria, the main modification sites are located in LPS and lipooligosaccharides (LOS). LPS is composed of lipid A, LPS core, and O-antigen, carrying negative charge (Figure 2 [Figure 2: see original paper]). LPS structure can not only maintain cellular balance but also form a physical barrier and defend against AMPs. Some LPS modifications depend on bacterial species and environmental factors to generate resistance to AMPs, among which lipid A modification is the most important.

1.3.1.1 Lipid A Modification Lipid A modifications can be divided into four categories: (1) Adding amino sugars. Amino sugars are usually aminoarabinose, glucosamine, and galactosamine. For example, *Acinetobacter baumannii*, *Francisella novicida*, and *Bordetella pertussis* modify lipid A by adding galactosamine and glucosamine. Adding glucosamine to lipid A in *Bordetella pertussis* can confer resistance to polymyxin B, colistin, LL-37, indolicidin, HHC-10, and CP28 [15]. (2) Phosphorylation and dephosphorylation. The *lpxF* gene encodes an enzyme that can remove a negatively charged phosphate from lipid A of Bacteroidetes LPS. This charge transformation makes it difficult for positively charged AMPs to bind to and disrupt the bacterial cell membrane. This makes the phosphate necessary for cell membrane construction become redundant when bacteria mature, thereby generating resistance to AMPs [16]. (3) Acylation and deacylation. Adding acyl groups to lipid A is considered an effective way to generate resistance to AMPs. The *pagP* gene can encode the addition of acyl groups to lipid A. *Bordetella parapertussis* with *pagP* gene mutation will be more susceptible to C18G than wild-type strains, while *P. aeruginosa* can develop resistance to C18G but not significantly to polymyxin B [17-18]. Moreover, the *pagP* gene can maintain membrane fluidity balance and repair membranes damaged by AMPs, which is another way to generate AMP resistance [19]. (4) Adding glycine. The *almEFG* operon can add glycine to lipid A of *Vibrio cholerae*, conferring resistance to polymyxin, though the exact mechanism remains unclear [20].

1.3.1.2 O-Antigen When *Burkholderia* symbiotically reproduces in the gut of the bean bug *Riptortus pedestris*, the loss of LPS O-antigen increases host AMP susceptibility [21]. The $\Delta wbaV$ gene mutation involved in O-antigen compilation in *Salmonella enterica* serovar Enteritidis increases its susceptibility to AMPs [22]. However, current research has not provided conclusive evidence that the presence and length of LPS O-antigen necessarily affect AMP resistance.

PG: phosphatidylglycerol; DPG: bisphosphatidylglycerol; Acyl-PG: acylphosphatidylglycerol; Lys-PG: lysine phosphatidylglycerol; Ala-PG: alanyl phosphatidylglycerol.

Figure 2 Gram-negative bacterial outer membrane (S. enterica)[1]

1.3.2 Gram-Positive Bacteria

Gram-positive bacteria lack outer membrane protection, so the cell wall bears all external pressure and defends against invasion of harmful molecules such as AMPs and antibiotics. Gram-positive bacterial cell walls contain large amounts of teichoic acids, which can be divided into two types: wall teichoic acid (WTA) and lipoteichoic acid (LTA). The former does not penetrate the plasma membrane, with its terminal end connected to peptidoglycan N-acetylmuramic acid residues via phosphodiester bonds, while LTA spans the peptidoglycan layer, with its terminal phosphate covalently connected to the oligosaccharide moiety of glycolipids in the plasma membrane.

LTA modification in Gram-positive bacteria is considered a universal way to generate resistance to AMPs, especially D-alanylation of LTA, while other LTA modifications such as adding choline phosphate and glycosylation have not been confirmed to confer AMP resistance [23]. The *dlt* operon is responsible for compiling D-alanine carrier protein ligase, which can add D-alanine to teichoic acids, increasing positive charge on the bacterial surface and improving resistance to AMPs [24]. Additionally, *Listeria monocytogenes* WTA contains L-rhamnosylation, which can delay the interaction between AMPs and the membrane by creating spatial hindrance, promoting AMP resistance formation [25].

2 Biofilm

2.1 Causes and Composition of Biofilms

Bacteria have evolved the ability to form multicellular structures. In clinical environments, bacteria are exposed to various adverse conditions such as antimicrobial agents, nutrient limitation, anaerobic conditions, and heat shock, which can stimulate bacterial stress responses. These responses, coordinated with other defense mechanisms, can form biofilms that enable bacterial survival in harsh environments. Biofilms are difficult to clear by host immune mechanisms and antimicrobial agents, making them one of the main factors for AMP resistance [26]. The transition from planktonic state to biofilm results from abnormal multiple regulatory networks caused by environmental changes, causing planktonic cells to contact a surface and form biofilms under sensing pressure signals, thereby gaining the ability to resist environmental changes [27-28].

Biofilms are composed of extracellular matrix, specific polysaccharides, proteins, water channels, and extracellular DNA (eDNA) [29]. Among them, extracellular polymeric substance (EPS) serves as the biofilm matrix, with channels inside the biofilm allowing water, air, and nutrients to pass through [30]. EPS components include: (1) Exopolysaccharides, which are high molecular weight polymers composed of sugar residues synthesized and secreted from inside or outside the cell to the external environment. They form linear or branched long chains attached to the cell surface and extend outward to form a huge network. Exopolysaccharides serve as a skeleton for other carbohydrates, proteins, nucleic acids, and lipids to attach [31]. (2) Extracellular proteins, another major

EPS component, can attach to cell and exopolysaccharide surfaces, contributing to biofilm formation and stability. (3) eDNA, secreted by lysed cells, is key for biofilm attachment. Its negative charge presents repulsion in existing attachments, but when bacteria are only a few nanometers from the surface, it interacts with underlying surface receptors to promote adhesion [32].

2.2 Biofilm Formation Process

The premise of biofilm formation is that bacteria are sufficiently close to a surface. When bacteria approach a surface, there are attractive and repulsive forces. At 10-20 nm from the surface, the negative charge on the bacterial surface and the negative charge on the environmental surface repel each other, but this repulsion is overcome by van der Waals forces between bacteria and the surface. Meanwhile, flagella and pili can help bacteria mechanically attach to the surface [33]. Once the biofilm matures, as the bacterial community develops, some cells detach from the original attached structure. This stage is called dispersal, which is key to completing the biofilm cycle [34]. Biofilm formation can be divided into three stages: attachment, maturation, and dispersal.

Attachment can be divided into two stages: reversible and irreversible attachment. Irreversibly attached biofilms can resist more intense physical and chemical attacks. In reversible attachment, flagella- and pili-mediated motility is very important. Flagella are key for initial bacterial contact with the surface, while pili can aggregate bacteria together to form colonies [35].

Mature biofilms trigger dispersal for various reasons, possibly due to nutrient deficiency, fierce competition, and overgrown bacterial communities. Dispersal can occur in part or the entire biofilm, releasing planktonic bacteria to form new biofilms at other sites [34].

2.3 Mechanisms of Biofilm Resistance to AMPs

AMPs can inhibit or eradicate biofilms in two ways [36]. (1) When AMP concentration equals or exceeds the minimal inhibitory concentration (MIC), AMPs can prevent biofilm formation by killing planktonic bacteria, kill bacteria detached from biofilms, and reduce or eradicate biofilms by killing bacteria inside them. (2) When AMP concentration is below MIC: a) Interfere with biofilm attachment by binding AMPs to material surfaces, bacterial surfaces, or EPS components to inhibit or prevent biofilm formation; b) Interfere with gene expression by controlling biofilm lifestyle types through interference with modifications, downregulating genes involved in matrix synthesis, abnormal regulation of other genes, and targeting signaling molecules such as stringent response ppGpp. Biofilms develop resistance to AMPs through structural barriers and induced hindrance.

Biofilms have stronger resistance to antimicrobial agents and active factors secreted by host immune systems than planktonic bacteria. It is estimated that

biofilm resistance to AMPs is 10,000 times that of planktonic bacteria [29]. However, bacterial resistance mechanisms to AMPs are not as thoroughly studied as other antimicrobial agents, and no specific bacterial resistance mechanisms to AMPs have been discovered, only some hypothetical mechanisms have been proposed (Figure 3 [Figure 3: see original paper]). Biofilm resistance pathways to AMPs can be divided into three categories: (1) Structural barriers: including extracellular matrix, heterogeneous bacterial communities, and efflux pumps; (2) Genetic control: including quorum sensing (QS), bis-(3' -5')-cyclic diguanosine monophosphate (c-di-GMP), and small RNAs (sRNAs); (3) Signal regulation: including two-component regulatory systems (TCS). Genetic control and signal regulation can be considered induced hindrance.

2.3.1 Structural Barriers EPS: Taking *P. aeruginosa* as an example, it synthesizes and releases three exopolysaccharides named alginate, glucose-rich polysaccharide (Pel), and pentasaccharide (Psl), which can make biofilms more stable. Alginate can interact with nutrients and water and provide them to the biofilm, while Pel and Psl support biofilm structure [38]. *P. aeruginosa* eDNA can confer resistance to AMPs by chelating cations, otherwise affecting biofilm structural function and the induction of PhoPQ and PmrAB regulatory factors [39]. Thus, exopolysaccharides mostly resist AMPs through isolation, while eDNA resists AMPs by binding to AMPs with different charges.

Heterogeneous bacterial communities: Biofilm bacterial communities include heterogeneous bacteria at different growth stages, and these differences are divided according to oxygen and nutrient gradients to determine whether bacteria should exist on the surface or deep inside the biofilm. Metabolically active bacteria are on the biofilm surface, while bacteria deprived of nutrients and oxygen are deep inside the biofilm, producing different stringent responses when exposed to different AMPs [26]. Among heterogeneous communities, one type of cells that can produce significant resistance is persister cells, which are far more numerous in biofilms than in planktonic bacteria. Persister cells have near-zero growth rates, and AMPs have almost no effect on them. Therefore, persister cells can serve as bacterial reservoirs, regrowing once antimicrobial pressure disappears. TN-5 can effectively resist persister cells of *P. aeruginosa* PDO300, not its biofilm [40]. Whether persister cells can transfer AMP killing of biofilms requires further research.

Efflux pumps: Efflux pump resistance to AMPs can be divided into planktonic and biofilm stages, but efflux pumps play a greater role in biofilms than in planktonic bacteria. Efflux pumps can expel AMPs and other antimicrobial agents from bacteria and biofilms. There are six families of efflux pumps: ABC, MFS, MATE, SMR, RND, and DMT [41]. Only ABC uses ATP as energy to expel AMPs, while other efflux pumps rely on electrochemical properties to expel AMPs.

2.3.2 Genetic Control Biofilm formation and dispersal are regulated by genes and environmental signals. The main known biofilm regulators are QS, c-di-GMP, and sRNAs [42]. (1) **QS:** When bacterial density reaches a certain level, bacteria secrete a self-produced signaling molecule called autoinducers. When autoinducer concentration reaches a threshold, target genes regulate the biofilm, and QS can also regulate eDNA, lectin, and biosurfactant production. Studies have found that *Burkholderia cenocepacia* QS regulates the expression of large amounts of surface proteins, lectins, and eDNA [43]. (2) **c-di-GMP:** As a second messenger regulating exopolysaccharide and surface protein synthesis and biofilm fluidity, two types of enzymes control c-di-GMP content in bacteria: DGGs and CGDEF [44]. *P. aeruginosa* c-di-GMP usually includes five parts: environmental signal sensor, enzymes involved in c-di-GMP synthesis and decomposition, specific effects (allosteric regulation by c-di-GMP regulatory proteins or riboswitches), target sites (DNA, enzymes, and molecular structures), and synthetic output [45]. (3) **sRNAs:** Non-coding small RNAs including riboswitches participate in post-transcriptional regulation of bacterial genes, participating in some metabolic pathways, stress adaptation, and pathogenesis [46]. The genetic control in *P. aeruginosa* biofilms is relatively well-studied, detailed in Table 1 .

Signal Regulation

TCS includes histidine kinase (HK) and response regulator (RR). HK is a sensor protein, usually composed of an N-terminal ligand-binding domain and a C-terminal kinase domain. Signal transduction occurs through phosphoryl group transfer, where a specific histidine residue is transferred from ATP to HK, and then HK transfers the phosphoryl group from the histidine residue to an aspartate residue on RR. This phosphate-activated RR activity is considered transcriptional regulation [47].

TCS involved in AMP resistance can be divided into four categories. (1) **PhoPQ TCS:** Widely present in *Salmonella typhimurium*, PhoQ acts as a sensor protein kinase to recognize low-level stimuli such as magnesium ions, acidic pH, and AMPs (polymyxin B, C18G, LL-37, and protegrin). Each stimulus promotes different levels of PhoP-regulated gene expression [48]. In *S. typhimurium*, PhoPQ TCS can control PgtE secretion to generate AMP resistance [49]; (2) **PmrAB TCS:** Mostly present in *S. typhimurium*, *Escherichia coli*, *K. pneumoniae*, *Yersinia pestis*, *Citrobacter rodentium*, and *P. aeruginosa*. PmrB can sense high concentrations of iron ions, magnesium ions, weak acid pH, and cationic AMPs [50]. PmrAB has good effect in resisting polymyxin B and easily obtains colistin-resistant strains [51]; (3) **CsrRS TCS:** Present in group A Streptococcus, CraS acts as a sensor protein kinase to recognize LL-37, RP-1, and polymyxin B [52]. Additionally, CsrRS TCS can control LPS modification in *Vibrio cholerae* by increasing glycine, with CraS promoting its regulation by directly binding to the regulatory region of the *almEFG* operon [53]; (4) **GacSA TCS:** Present in *P. aeruginosa* and *S. typhimurium*, promoting biofilm formation [38].

TCS often has cross-talk. In *E. coli*, PhoPQ TCS and PmrAB TCS have cross-talk mediated by PmrD, but PmrD activation does not depend on PhoP/PhoQ, demonstrating the complex process of TCS [54]. Additionally, TCS can work synergistically with efflux pumps. Gram-positive bacteria have a special structure called peptide sensing and detoxification modules (PSD), which results from TCS recognition of AMPs and ABC efflux of AMPs [55].

Signal regulation also includes the following three parts. (1) **Stringent response:** When bacteria grow in nutrient-poor environments (such as lacking amino acids), cellular protein synthesis and other metabolic activities are shut down, where ppGpp is key to mediating the stringent response, which can induce toxin-antitoxin (TA) responses to control bacterial persistence [56]. (p)ppGpp is linked to transcriptional activation of toxin HokB through Obg. Obg can control persistence in *P. aeruginosa* biofilms by inducing HokB expression, while increased HokB levels cause biofilm polarization and dormancy [57]. (2) **TA model:** The TA model is key to inducing persistence. Class 2 TA models usually consist of two proteins: a toxin that inhibits important cellular functions and an antitoxin composed of the toxin that inactivates it. During stress responses, the antitoxin is degraded into free toxin, which can hinder DNA transcription and translation, AMPs, or cell wall synthesis [41]. (3) **SOS response:** Can repair damaged DNA, preventing AMPs from causing mutations in bacterial key genes, thereby generating resistance to AMPs.

Gram-positive bacteria mostly resist AMP invasion through thicker peptidoglycan layers and altered surface charge, also secreting some proteases or molecules attached to the bacterial surface to eliminate or reduce AMPs. Gram-negative bacteria mainly generate AMP resistance through outer membrane modifications. Additionally, bacteria can secrete vesicles or extracellular polysaccharides and proteins to trap AMPs, and efflux pumps also prevent AMPs from approaching the bacterial cell membrane.

When planktonic bacteria reach a certain population, they will form biofilms, and the resulting induced hindrance and structural barriers will generate greater resistance to AMPs. Currently, research on bacterial resistance to AMPs remains at the laboratory stage, while extensive clinical trials have proven that bacterial tolerance caused by AMPs is much milder than traditional antibiotics. Therefore, AMPs still have broad prospects as a new generation of antimicrobial drugs, but the possibility of future resistance development should also receive sufficient attention. Only by considering both AMP bacteriostatic mechanisms and bacterial resistance mechanisms to AMPs can we design highly effective AMPs. During production, large-scale use and abuse should be strictly controlled to maximize advantages and benefit humanity.

References

[1] NURI R,SHPRUNG T,SHAI Y.Defensive remodeling:how bacterial surface properties and biofilm formation promote resistance to antimicrobial pep-

tides[J].Biochimica et Biophysica Acta: Biomembranes,2015,1848(11):3089-3100.

[2] MARIA-NETO S,DE ALMEIDA K C,MACEDO M L R,et al.Understanding bacterial resistance to antimicrobial peptides:from the surface to deep inside[J].Biochimica et Biophysica Acta (BBA)-Biomembranes,2015,1848(11):3078-3088.

[3] NGUYEN L T,HANEY E F,VOGEL H J.The expanding scope of antimicrobial peptide structures and their modes of action[J].Trends in Biotechnology,2011,29(9):464-472.

[4] OKUMURA C Y M,NIZET V.Subterfuge and sabotage:evasion of host innate defenses by invasive gram-positive bacterial pathogens[J].Annual Review of Microbiology,2014,68(1):439-458.

[5] LLOBET E,TOMÁS J M,BENGOECHEA J A.Capsule polysaccharide is a bacterial decoy for antimicrobial peptides[J].Microbiology,2008,154(12):3877-3886.

[6] CAMPOS M A,VARGAS M A,REGUEIRO V,et al.Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides[J].Infection and Immunity,2004,72(12):7107-7114.

[7] FLEITAS O,AGBALE C M,FRANCO O L.Bacterial resistance to antimicrobial peptides:an evolving phenomenon[J].Frontiers in Bioscience,2016,21(5):1013-1038.

[8] SMYTH D,CAMERON A,DAVIES M R,et al.DrsG from Streptococcus dysgalactiae subsp.equisimilis inhibits antimicrobial peptide LL-37[J].Infection Immunity,2014,82(6):2337-2344.

[9] ROLIN O,MUSE S J,SAFI C,et al.Enzymatic modification of lipid A by ArnT protects Bordetella bronchiseptica against cationic peptides and required transmission[J].Infection and Immunity,2014,82(2):491-499.

[10] BAYER A S,MISHRA N N,SAKOULAS G,et al.Heterogeneity of mprF sequences in methicillin-resistant Staphylococcus aureus clinical isolates:role in cross-resistance between daptomycin defense antimicrobial peptides[J].Antimicrobial Agents Chemotherapy,2014,58(12):7462-7467.

[11] COX E,MICHALAK A,PAGENTINE S,et al.Lysylated phospholipids stabilize models of bacterial lipid bilayers and protect against antimicrobial peptides[J].Biochimica et Biophysica Acta: Biomembrane,2014,1838(9):2198-2204.

[12] ERNST C M,KUHN S,SLAVETINSKY C J,et al.The lipid-modifying multiple peptide resistance factor oligomer consisting distinct interacting synthase flippase subunits[J].mBio,2015,6(1):e02340-14.

[13] KUMARIYA R,SOOD S K,RAJPUT Y S,et al.Increased membrane surface positive charge and altered membrane fluidity leads to cationic antimicrobial

peptide resistance in Enterococcus faecalis[J].Biochimica et Biophysica Acta: Biomembrane,2015,1848(6):1367-1375.

[14] LATHER P,MOHANTY A K,JHA P,et al.Changes associated with cell membrane composition of Staphylococcus aureus on acquisition of resistance against class a bacteriocin and its in vitro substantiation[J].European Food Research and Technology,2015,240(1):101-107.

[15] SHAH N R,HANCOCK R E W,FERNANDEZ R C.Bordetella pertussis lipid A glucosamine modification confers resistance to cationic antimicrobial peptides and increases resistance to outer membrane perturbation[J].Antimicrobial Agents and Chemotherapy,2014,58(8):4931-4934.

[16] CULLEN T W,SCHOFIELD W B,BARRY N A,et al.Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation[J].Science,2015,347(6218):170-175.

[17] HITTLE L E,JONES J W,HAJJAR A M,et al.Bordetella parapertussis PagP mediates the addition of two palmitates to the lipopolysaccharide lipid A[J].Journal of Bacteriology,2015,197(3):572-580.

[18] THAIPISUTTIKUL I,HITTLE L E,CHANDRA R,et al.A divergent Pseudomonas aeruginosa palmitoyltransferase essential cystic fibrosis-specific lipid A[J].Molecular Microbiology,2014,91(1):158-174.

[19] BAND V I,WEISS D S.Mechanisms of antimicrobial peptide resistance in gram-negative bacteria[J].Antibiotics,2015,4(1):18-41.

[20] HENDERSON J C,FAGE C D,CANNON J R,et al.Antimicrobial peptide resistance of vibrio cholerae results from an LPS modification pathway related to nonribosomal peptide synthetases[J].ACS Chemical Biology,2014,9(10):2382-2392.

[21] KIM J K,SON D W,KIM C H,et al.Insect gut symbiont susceptibility to host antimicrobial peptides caused alteration bacterial envelope[J].The Journal of Biological Chemistry,2015,290(34):21042-21053.

[22] JAISWAL S,PATI N B,DUBEY M,et al.The O-antigen negative ΔwbaV mutant of Salmonella enterica serovar Enteritidis shows adaptive resistance to antimicrobial peptides and elicits colitis in streptomycin pretreated mouse model[J].Gut Pathogens,2015,7(1):24.

[23] PERCY M G,GRÜNDLING A.Lipoteichoic acid synthesis and function in gram-positive bacteria[J].Annual Review of Microbiology,2014,68(1):81-100.

[24] NEUHAUS F C,BADDILEY J.A continuum of anionic charge:structures and functions of D-alanyl-teichoic acids in gram-positive bacteria[J].Microbiology and Molecular Biology Reviews,2003,67(4):686-723.

[25] CARVALHO F,ATILANO M L,POMBINHO R,et al.L-rhamnosylation of Listeria monocytogenes wall teichoic acids promotes resistance to an-

timicrobial peptides by delaying interaction with the membrane[J].PLoS Pathogens,2015,11(5):e1004919.

[26] DE LA FUENTE-NÚÑEZ C,REFFUVEILLE F,FERNÁNDEZ L,et al.Bacterial biofilm development a multicellular adaptation:antibiotic resistance therapeutic strategies[J].Current Opinion in Microbiology,2013,16(5):580–589.

[27] HALL-STOODLEY L,COSTERTON J W,STOODLEY P.Bacterial biofilms:from the natural environment to infectious diseases[J].Nature Reviews Microbiology,2004,2(2):95–108.

[28] COSTERTON J W,STEWART P S,GREENBERG E P.Bacterial biofilms:a common cause of persistent infections[J].Science,1999,284(5418):1318–1322.

[29] RABIN N,ZHENG Y,OPOKU-TEMENG C,et al.Biofilm formation mechanisms and targets for developing antibiofilm agents[J].Future Medicinal Chemistry,2015,7(4):493–512.

[30] FLEMMING H C,NEU T R,WOZNIAK D J.The EPS matrix:the "house of biofilm cells" [J].Journal of Bacteriology,2007,189(22):7945–7947.

[31] NWODO U U,GREEN E,OKOH A I.Bacterial exopolysaccharides:functionality and prospects[J].International Journal of Molecular Sciences,2012,13(11):14002–14015.

[32] DAS T,SHARMA P K,BUSSCHER H J,et al.Role of extracellular DNA in initial bacterial adhesion surface aggregation[J].Applied Environmental Microbiology,2010,76(10):3405–3408.

[33] PALMER J,FLINT S,BROOKS J.Bacterial cell attachment,the beginning of a biofilm[J].Journal of Industrial Microbiology & Biotechnology,2007,34(9):577–588.

[34] O' TOOLE G,KAPLAN H B,KOLTER R.Biofilm formation as microbial development[J].Annual Review of Microbiology,2000,54(1):49–79.

[35] RENNER L D,WEIBEL D B.Physicochemical regulation of biofilm formation[J].MRS Bulletin,2011,36(5):347–355.

[36] BATONI G,MAISETTA G,ESIN S.Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria[J].Biochimica et Biophysica Acta: Biomembrane,2016,1858(5):1044–1060.

[37] RIBEIRO S M,FELÍCIO M R,BOAS E V,et al.New frontiers for anti-biofilm drug development[J].Pharmacology & Therapeutics,2016,160:133–144.

[38] RASAMIRAVAKA T,LABTANI Q,DUEZ P,et al.The formation of biofilms by Pseudomonas aeruginosa:a review of the natural and synthetic compounds interfering with control mechanisms[J].Biomed Research International,2015,2015:759348.

[39] RODRÍGUEZ-ROJAS A,MAKAROVA O,MÜLLER U,et al.Cationic peptides facilitate iron-induced mutagenesis in bacteria[J].PLoS Genetics,2015,11(10):e1005546.

[40] BAHAR A A,LIU Z G,GARAFALO M,et al.Controlling persister and biofilm cells of gram-negative bacteria with a new 1,3,5-triazine derivative[J].Pharmaceuticals,2015,8(4):696-710.

[41] VAN ACKER H,COENYE T.The role of efflux and physiological adaptation in biofilm tolerance and resistance[J].The Journal of Biological Chemistry,2016,291(24):12565-12572.

[42] WOLSKA K I,GRUDNIAK A M,RUDNICKA Z,et al.Genetic control of bacterial biofilms[J].Journal of Applied Genetics,2016,57(2):225-238.

[43] FAZLI M,ALMBLAD H,RYBTKE M L,et al.Regulation of biofilm formation in Pseudomonas and Burkholderia species[J].Environmental Microbiology,2014,16(7):1961-1981.

[44] RÖMLING U,GALPERIN M Y,GOMELSKY M.Cyclic di-GMP:the first 25 years of a universal bacterial second messenger[J].Microbiology and Molecular Biology Reviews,2013,77(1):1-52.

[45] HENGGE R.Principles c-di-GMP signalling bacteria[J].Nature Reviews Microbiology,2009,7(4):263-273.

[46] KALIA V C,WOOD T K,KUMAR P.Evolution of resistance to quorum-sensing inhibitors[J].Microbial Ecology,2014,68(1):13-23.

[47] BOLES B R,THOENDEL M,ROTH A J,et al.Identification of genes involved polysaccharide-independent Staphylococcus aureus biofilm formation[J].PLoS One,2010,5(4):e10146.

[48] GROISMAN E A,MOUSLIM C.Sensing by bacterial regulatory systems in host and non-host environments[J].Nature Reviews Microbiology,2006,4(9):705-709.

[49] LIU Y H,ZHANG Q F,HU M,et al.Proteomic analyses of intracellular Salmonella enterica serovar typhimurium reveal extensive bacterial adaptations to infected host epithelial cells[J].Infection and Immunity,2015,83(7):2897-2906.

[50] CHEN H D,GROISMAN E A.The biology of the PmrA/PmrB two-component system:the major regulator of lipopolysaccharide modifications[J].Annual Review of Microbiology,2013,67(1):83-112.

[51] 葛琳, 郭大伟, 何方, 等.PmrA-PmrB 二元调控系统介导大肠杆菌对黏杆菌素耐药的机制研究 [J]. 畜牧兽医学报,2016,47(4):812-819.

[52] CHEUNG A L,BAYER A S,YEAMAN M R,et al.Site-specific mutation of the sensor kinase GraS in Staphylococcus aureus alters the adaptive response to distinct cationic antimicrobial peptides[J].Infection and Immunity,2014,82(12):5336-5345.

[53] BILECEN K,FONG J C N,CHENG A,et al.Polymyxin B resistance and biofilm formation in Vibrio cholerae controlled response regulator CarR[J].Infection Immunity,2015,83(3):1199-1209.

[54] RUBIN E J,HERRERA C M,CROFTS A A,et al.PmrD is required for modifications to Escherichia coli endotoxin that promote antimicrobial resistance[J].Antimicrobial Agents and Chemotherapy,2015,59(4):2051-2061.

[55] DINTNER S,HEERMANN R,FANG C,et al.A sensory complex consisting of an ATP-binding cassette transporter and a two-component regulatory system controls bacitracin resistance in Bacillus subtilis[J].The Journal of Biological Chemistry,2014,289(40):27899-27910.

[56] MAISONNEUVE E,CASTRO-CAMARGO M,GERDES K.(p)ppGpp controls bacterial persistence by stochastic induction of toxin-antitoxin activity[J].Cell,2013,154(5):1140-1150.

[57] VERSTRAETEN N,KNAPEN W J,KINT C I,et al.Obg and membrane depolarization are part of a microbial bet-hedging strategy that leads to antibiotic tolerance[J].Molecular Cell,2015,59(1):9-21.

Figure 1 The action mechanism of AMPs[3]

Figure 3 Mechanism of biofilm resistance to AMPs[26,37]

Note: Figure translations are in progress. See original paper for figures.