PHILOSOPHICAL TRANSACTIONS B

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Review

**Cite this article:** Joo H-S, Fu C-I, Otto M. 2016 Bacterial strategies of resistance to antimicrobial peptides. *Phil. Trans. R. Soc. B* **371**: 20150292.
http://dx.doi.org/10.1098/rstb.2015.0292

Accepted: 18 January 2016

One contribution of 13 to a theme issue 'Evolutionary ecology of arthropod antimicrobial peptides'.

**Subject Areas:**
microbiology

**Keywords:**
bacterial resistance, antimicrobial peptides, *Staphylococcus*

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# Bacterial strategies of resistance to antimicrobial peptides

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Antimicrobial peptides (AMPs) are a key component of the host's innate immune system, targeting invasive and colonizing bacteria. For successful survival and colonization of the host, bacteria have a series of mechanisms to interfere with AMP activity, and AMP resistance is intimately connected with the virulence potential of bacterial pathogens. In particular, because AMPs are considered as potential novel antimicrobial drugs, it is vital to understand bacterial AMP resistance mechanisms. This review gives a comparative overview of Gram-positive and Gram-negative bacterial strategies of resistance to various AMPs, such as repulsion or sequestration by bacterial surface structures, alteration of membrane charge or fluidity, degradation and removal by efflux pumps.

This article is part of the themed issue 'Evolutionary ecology of arthropod antimicrobial peptides'.

## 1. Introduction

Antimicrobial peptides (AMPs) represent an important part of innate immune defences in many organisms [1]. They contribute to immune defences on epithelial surfaces and form part of the variety of antimicrobial agents by which leucocytes kill microorganisms after ingestion [2]. Many AMPs have activity against a wide range of pathogens. In addition to their antimicrobial activity, AMPs also have a signalling function. For example, they can activate components of the human acquired immune system, such as T cells and dendritic cells [3].

AMPs are synthesized as proforms before processing to the active, mature peptides occurs. Most AMPs are cationic (cationic AMPs, CAMPs) and show pronounced amphipathy. Although for many AMPs the mode of action is incompletely understood, these features contribute to binding to the anionic bacterial surface and integration into the cytoplasmic membrane, where many AMPs form pores to kill the target microorganism [4].

Most AMPs in humans belong to the beta-defensin family. Human beta-defensins 1–4 are produced by keratinocytes on the skin [5]. The only member of the cathelicidin family produced in humans is the LL-37 peptide [6]. While these peptides are cationic, humans also produce an anionic AMP, dermcidin, which recently has been shown to also work by pore formation in target membranes [7,8].

AMPs have often been suggested as potential novel antimicrobial compounds [9]. Notably, their commonly bactericidal mode of action makes them potential candidates for difficult-to-treat infections by slow-growing bacteria, such as biofilm infections. As the development of resistance to antimicrobial compounds represents one of the most serious problems for healthcare systems, a detailed understanding of resistance mechanism to AMPs is of vital importance.

AMP resistance mechanisms include proteolytic degradation or sequestration by secreted proteins, impedance by exopolymers and biofilm matrix molecules, circumvention of attraction by cell surface/membrane alteration, and export by efflux pumps. This review gives an overview of each mechanism in Gram-positive and Gram-negative bacteria and finishes by a presentation of mechanisms bacteria

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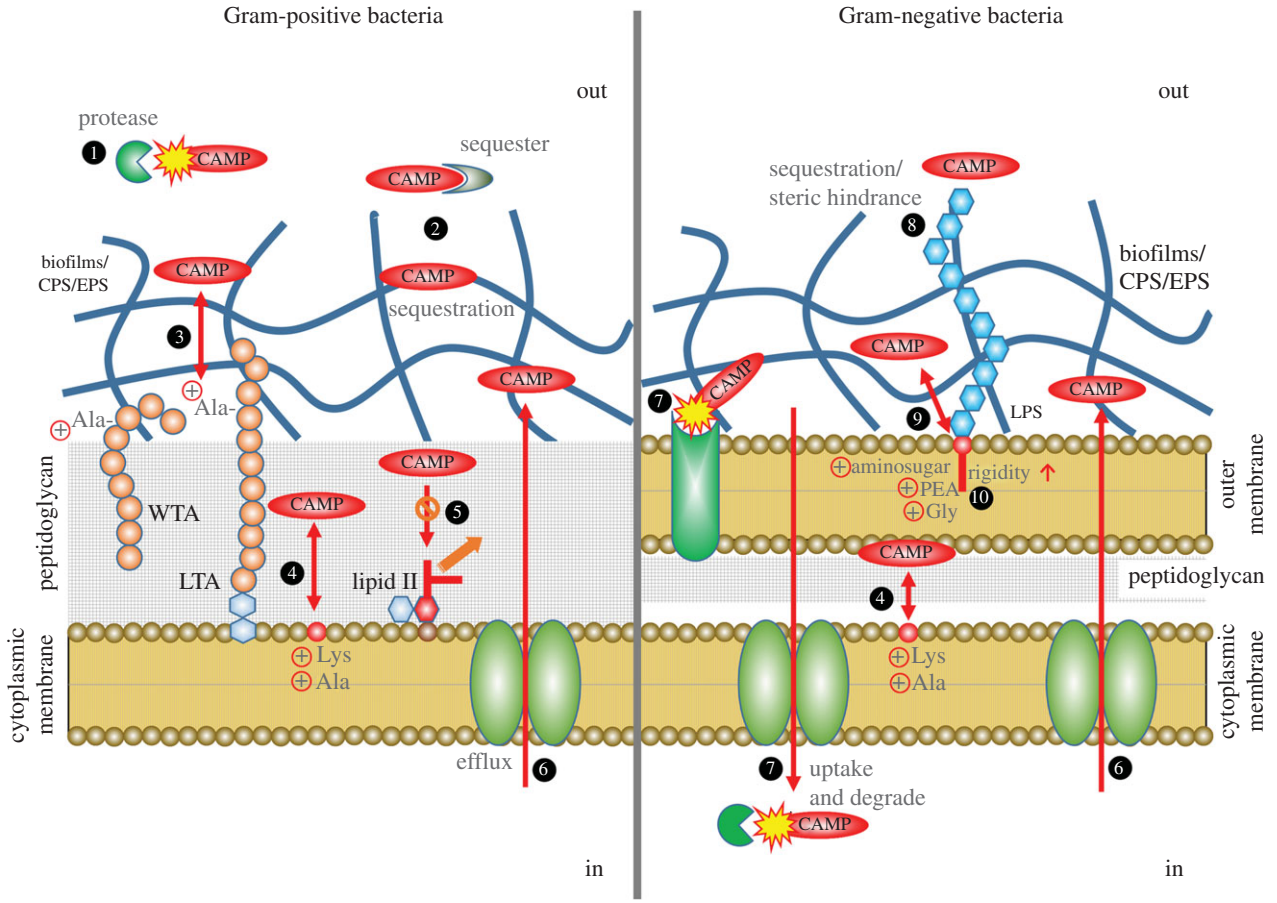


**Figure 1.** Schematic diagram of bacterial AMP resistance mechanisms. Mechanisms in Gram-positive bacteria are shown on the left, and in Gram-negative bacteria on the right. Gram-positive bacteria retain the Gram stain owing to their thick peptidoglycan cell wall, while Gram-negative bacteria only have a thin peptidoglycan (PG) layer but possess an additional so-called 'outer membrane'. 1, Proteolytic degradation by extracellular proteases; 2, sequestration by extracellular proteins or extracellular matrix; 3, electrostatic repulsion by alanylated teichoic acids (TA); 4, electrostatic repulsion by aminoacylated PG; 5, evasion of lipid II-binding AMPs by pentapeptide alteration; 6, export of AMPs by efflux pumps; 7, proteolytic cleavage by outer membrane protease or cytosolic protease after uptake by transporters; 8, sequestration or steric hindrance by O-antigen of lipopolysaccharide (LPS); 9, electrostatic repulsion by amine compound-added lipid A; 10, enhanced rigidity by lipid A acylation.

**Table 1.** Overview of bacterial resistance mechanisms against antimicrobial peptides.

| mechanisms | Gram-positive bacteria | Gram-negative bacteria |
|---|---|---|
| extracellular proteins | proteolytic degradation | proteolytic degradation |
| | sequestration | |
| exopolymers | PIA, PGA | alginate, polysialic acid |
| surface modification | repulsion by D-alanylation of TA | repulsion by lipid A phosphate modification |
| | steric hindrance by L-rhamnosylation of WTA | increased OM rigidity by lipid A acylation |
| | lipid II modification | O-antigen of LPS |
| cytoplasmic membrane alteration | charge repulsion by PG amino-acylation | increased IM rigidity by PG acylation |
| efflux pumps | export by ABC transporters | export by RND family efflux pumps |

have to sense the presence of AMPs (see table 1 and figure 1 for a summary list and graphical presentation, respectively).

## 2. Extracellular proteins

Secreted bacterial proteins, such as proteases, are the first bacterial defence mechanisms that AMPs encounter when interacting with bacteria. Proteolytic degradation of AMPs by extracellular enzymes represents a simple, yet effective way of providing AMP resistance to microorganisms. Commensal bacteria that live on mammalian epithelial surfaces, such as staphylococci, secrete various proteases. In staphylococci, these include metalloproteases such as aureolysin and SepA, and serine endopeptidases such as the V8 protease, which are known to degrade linear CAMPs such as the human cathelicidin LL-37 [10,11]. Group A *Streptococcus* produces a protease called SpeB. This cysteine protease has been shown to fragmentize many host AMPs, including LL-37 and beta-defensins [12–15]. Interestingly, exploiting host proteins can intensify

the proteolytic activity of SpeB. Namely, interaction of SpeB with cell wall-anchored G-related alpha2M-binding (GRAB) protein leads to a surface-bound complex of SpeB and a host proteinase inhibitor, alpha2-macroglobulin, which shows increased activity towards LL-37 [16,17]. In addition, as a secondary effect of SpeB proteolytic activity, degraded host proteoglycans release dermatan sulfate, which completely neutralizes human alpha-defensin, HNP-1 [18]. Finally, proteases from another Gram-positive pathogen, *Enterococcus faecalis*, and the Gram-negative *Pseudomonas aeruginosa* and *Proteus mirabilis* have also been reported to degrade LL-37 [12].

One of the most intensively studied group of proteases in Gram-negative bacteria is the omptin family, a group of aspartate proteases found in the enterobacterial outer membrane (OM) [19,20]. Some representative members of that protease, such as OmpT in *Escherichia coli*, PgtE in *Salmonella enterica* serotype Typhimurium (*S. typhimurium*) and Pla in *Yersinia pestis*, have been demonstrated to cleave AMPs including LL-37, the homologous murine cathelicidin-related antimicrobial peptide (CRAMP) and protamine [21–23]. Along with the opmtin family of proteases, metalloproteases play an important role in the defence of Gram-negative bacteria towards AMPs. For example, the metalloprotease ZapA of *P. mirabilis* neutralizes multiple targets such as LL-37, human beta-defensin HBD-1, and the porcine AMP protegrin-1 [24]. *Burkholderia cenocepacia* produces two metalloproteases, ZmpA and ZmpB, which can degrade various CAMPs [25]. Interestingly, ZmpA cleaves linear LL-37, whereas ZmpB targets the nonlinear HBD-1 [25]. Some Gram-negative bacteria also degrade AMPs in the intracellular environment after import by specific transport proteins. *S. typhimurium* and *Haemophilus influenzae* produce an ABC transporter encoded by the *sapABCDFZ* operon, which was reported to increase bacterial resistance to CAMPs [26–28]. Binding of CAMPs by SapA leads to increased expression of the genes in the *sap* operon, whose four-component ABC transporter product transfers CAMPs into the cytosol for intracellular proteolytic degradation [28–30]. *H. influenzae* SapA has been shown to bind to diverse AMPs such as LL-37, HNP-1, HBD-2, HBD-3 and melittin [31].

Finally, it needs to be stressed that the inactivation of AMPs by proteases is highly dependent on the structure of the target peptide [1]. For example, a linear AMP such as LL-37 is more prone to degradation than AMPs with nonlinear structures that contain disulfide bonds. The introduction of disulfide bonds, or the even more sophisticated post-translational modification found in some bacterial AMPs (bacteriocins) such as lantibiotics represent resistance mechanisms of AMPs towards proteolytic degradation by the host or competing microorganisms, exemplifying the multi-faceted evolutionary interplay of AMP producers and resistant microorganisms [1,32].

Along with proteolysis, sequestration has an important role in extracellular protein-mediated resistance to AMPs. Staphylokinase is one of the most prominent extracellular AMP-sequestering molecules. It is encoded by the *sak* gene in *Staphylococcus aureus* and sequesters alpha-defensins (HNP-1 and 2) [33,34]. Furthermore, binding to LL-37 increases staphylokinase-dependent plasminogen activation, which represents another crucial role of staphylokinase in pathogenesis [35]. Intriguingly, the activation of plasminogen by streptokinase (Ska) of group A streptococci, in addition to the original activities of plasmin such as fibrin clot degradation [36], leads to the destruction of LL-37 [37]. Another well-known streptococcal sequester, streptococcal inhibitor of complement (SIC), protects *Streptococcus pyogenes* from defensins and LL-37 [38] as well as the membrane attack complex [39]. Finally, some streptococcal cell surface-attached proteins such as the M1 protein of *S. pyogenes* and the PilB pilin subunit of *Streptococcus agalactiae* can bind cathelicidins (LL-37 and CRAMP), contributing to streptococcal AMP resistance [40,41].

## 3. Biofilms and exopolymers

Bacterial biofilm is a consortium of surface-attached bacterial cells that are embedded in a matrix composed mainly of extracellular proteins, extracellular DNA and exopolysaccharides (EPS) [42,43]. Bacteria in biofilms exhibit higher resistance (up to 1000-fold) to antibiotics and AMPs than planktonic bacteria [44,45]. The increased resistance is thought to be partly owing to the decreased penetration of AMPs through the matrix [46]. EPS and capsular polysaccharides (CPS) impede AMPs by capturing or repelling them. Polysaccharide intercellular adhesin (PIA, also known as poly-*N*-acetyl glucosamine), which is produced by *S. aureus*, *Staphylococcus epidermidis*, and a variety of other bacteria including other staphylococci and *E. coli*, is responsible for the resistance to cationic HBD-3 and LL-37 as well as anionic dermcidin [47,48]. IcaB-mediated deacetylation of PIA increases the positive net charge in PIA. This results in increased repulsion to CAMPs, but perhaps also increased sequestration of dermcidin, in addition to forming a mechanical barrier for both types of AMPs [49]. Additionally, PIA is crucial for *in vitro* and *in vivo* biofilm formation, thus contributing to biofilm formation as a general AMP resistance mechanism [49–51]. On the other hand, impedance of CAMPs such as polymyxin B, HNP-1, HBD-1, lactoferrin and protamine by the anionic CPS of *Klebsiella pneumoniae*, *Streptococcus pneumoniae* or *P. aeruginosa* is presumably the result of structural hindrance as well as electrostatic trapping [52,53]. Capsules in other streptococci also have an important role in sequestering AMPs. Hyaluronic acid capsule as well as M protein of group A streptococci mediate resistance to LL-37 [54]. Capsule synthesized by a phosphoglucomutase homologue in *Streptococcus iniae* has a role in resistance to the fish AMP, moronecidin [55]. Furthermore, as briefly mentioned in §2, *P. aeruginosa*, *E. faecalis* and *S. pyogenes* can exploit host polysaccharide to sequester AMPs after the degradation of the host proteoglycan matrix by secreted bacterial proteases [18].

In *Pseudomonas* species, alginate is a major component of the biofilm matrix. It is an acylated polysaccharide comprising anionic sugars such as guluronic acid and mannuronic acid [56,57], whose role in AMP resistance in biofilms was shown by genetic overexpression [58]. Mechanisms of resistance to LL-37 in *P. aeruginosa* and other lung pathogens are largely dependent on sequestration by EPS [59,60]. Finally, the Gram-negative bacterium, *Neisseria meningitidis*, has a capsular polysialic acid that prevents surface binding of defensins, cathelicidins, protegrins and polymyxin B [61,62].

Poly-gamma-glutamic acid (PGA), which is a homopolymer of glutamic acid linked by gamma-amide bonds, forms an extracellular capsule protecting bacteria from phagocytosis [63]. PGA is found only in Gram-positive bacteria, mainly in *Bacillus* species and coagulase-negative staphylococci including *S. epidermidis* [64,65]. In addition to its role in resisting ingestion by leucocytes, PGA provides protection from LL-37, HBD-3 and dermcidin, similar to PIA. However,

in contrast to PIA, PGA does not directly facilitate biofilm formation [64,66], although the PGA biosynthesis *cap* operon is upregulated in biofilms of *S. epidermidis* [67,68].

Finally, it is important to note that AMPs have gained much attention as alternatives to traditional antibiotics in the treatment of biofilm-associated infection. This is mainly because the mode of action of AMPs is usually bactericidal, whereas many traditional antibiotics are commonly bacteriostatic and target fast-growing bacteria, thus lacking efficiency against biofilms [68,69]. However, despite numerous efforts of biofilm control with natural or synthetic AMPs, biofilm-intrinsic AMP resistance complicates the use of AMPs for the treatment of biofilm infections [70,71].

## 4. Surface modification

The bacterial cell envelope represents a major impediment for AMP activity. Because Gram-positive and Gram-negative bacteria have distinct cell envelope structures, they apply different strategies to modify their cell surface to resist AMPs. The key molecules for this purpose are anionic polymers attached to the outermost cell surface, teichoic acids (TA) in the Gram-positive cell wall and lipopolysaccharide (LPS) in the Gram-negative OM.

TA is the most abundant component in the Gram-positive cell wall, representing over 60% of its total mass [72]. It is composed of disaccharide anchors and phosphodiester-linked polyglycerol phosphate or polyribitol phosphate, which is responsible for the negative net charge of TA [73,74]. According to the anchoring location of TA to the cell surface, one distinguishes wall teichoic acid (WTA) and lipoteichoic acid. D-Alanylation on free hydroxyls of the repeating sugars, driven by the gene products of the *dltABCD* locus, adds a positive charge to TA. This generally lowers attraction of CAMPs and was shown in *S. aureus* for HNP-1,2,3, protegrins, magainin II, gallidermin and nisin [75]. In a more recent study on group B *Streptococcus*, it was shown that D-alanylation also increases cell wall density, which suggests a dual role of DltABCD, leading to electrostatic repulsion and a reduction of surface permeability [76]. D-Alanylation of TA as an AMP resistance strategy is widely used by various Gram-positive bacteria, including *Staphylococcus, Streptococcus* and *Bacillus* [75–83]. Interestingly, the Gram-negative bacterium, *Bordetella pertussis*, has a Dlt homologue, Dra, which leads to D-alanylation on the OM, resulting in a decreased negative charge that increases resistance to AMPs such as LL-37, HNP-1, HNP- 2 and polymyxin B [84]. Although the target component in the OM is yet to be determined, this finding suggests that Dlt-homologous proteins may have an even more widespread role in AMP resistance than previously believed. Recently, it was reported that L-rhamnosylation of WTA in *L. monocytogenes* protects from AMPs such as cathelicidins and gallidermin by increasing steric hindrance in the cell wall, proposing yet another TA decoration-related resistance mechanism [85].

Owing to its pivotal role in cell proliferation, the bacterial peptidoglycan precursor, lipid II, represents a further prominent AMP target. Lipid II is a cell wall building block consisting of undecaprenyl pyrophosphate and pentapeptide connected to the cell wall disaccharide of *N*-acetyl muramic acid (MurNAc) and *N*-acetyl glucosamine (GlcNAc) [86]. Many bacteria modify lipid II to evade AMP killing. Of note, unlike AMP-induced envelope alteration, modification of lipid II is a consistent change. The best-known lipid II modification is replacement of the terminal D-alanine to evade the activity of the glycopeptide antibiotic, vancomycin. Vancomycin binds to the D-Ala–D-Ala dipeptide found in the cell wall-cross-linking pentapeptide bridge and blocks the transpeptidation reaction that is required for cell wall synthesis [87]. Vancomycin-resistant strains contain lipid II with D-lactate or D-serine instead of the terminal D-alanine and show 1000-fold increased resistance [88]. Another well-known group of AMPs targeting lipid II include the bacterial lantibiotic-type bacteriocins, such as nisin or epidermin, which also use lipid II as docking molecule for subsequent pore formation [89,90]. In addition to vancomycin and lantibiotics, the human AMPs HNP-1 and HBD-3 target lipid II to block cell wall biosynthesis [91,92]. Finally, there are reports of resistance to lysozyme that is conferred by acetylation of MurNAc [93] or deacetylation of GlcNAc [94].

Similar to Gram-positive bacteria, Gram-negative bacteria accomplish resistance to AMPs by alteration of net charge and permeability of the cell surface. The counterpart of TA in Gram-negative bacteria is LPS, which forms the main component of the outer leaflet of the OM, covering 75% of the cell surface [95] and causing a net negative surface charge. Prevention of electrostatic binding of AMPs to the Gram-negative cell surface is achieved by amine-containing molecules (amino sugars, phosphoethanolamine (PEA) or glycine), which increase the positive charge of the anionic LPS component lipid A. *P. aeruginosa* and *S. typhimurium* attach aminoarabinose to a phosphate group in lipid A [96,97]. *Acinetobacter baumannii*, *Francisella novicida* and *Bordetella* species modify lipid A phosphate with galactosamine or glucosamine [98–101]. Furthermore, Gram-negative bacteria use PEA to decrease the anionic properties of LPS. *S. typhimurium*, *Neisseria gonorrhoeae* and *A. baumannii* attach PEA onto phosphates in lipid A [101–103]. Finally, *Vibrio cholerae* imports glycines into lipid A acyl chains to increase the positive charge and diminish AMP attraction [104]. Especially *Burkholderia* species and *N. meningitidis* constitutively express aminoarabinose- and PEA-attached lipid A, respectively; thus, inherently they have higher AMP resistance than other bacteria [105,106]. In addition to adding positively charged molecules to LPS, Gram-negative bacteria can obtain the same result by removing anionic phosphate groups from lipid A. In *F. novicida*, the LpxE and LpxF proteins have phosphatase activities, catalysing the removal of phosphate from the 1 and 4′ position of lipid A, respectively [107,108]. In contrast, LpxT is responsible for additional phosphorylation on lipid A [109,110]. Activation of LpxE/LpxF and inhibition of LpxT may contribute to AMP resistance in Gram-negative bacteria by decreasing the net negative charge [111–113]. Finally, it has recently been discovered that LPS dephosphorylation by LpxF is critical for survival of the human gut commensal *Bacteroidetes*, by protecting this bacterium from inflammatory perturbation originating from AMPs [114].

Another strategy of increasing AMP resistance in Gram-negative bacteria is to enhance the rigidity of the OM by adding extra acyl chains into lipid A [115,116]. Lipid A acylation is often mediated by the PagP enzyme, which was shown to promote resistance to various AMPs such as C18G, protegrin, polymyxin B, LL-37 and magainin II in *S. typhimurium*, *E. coli*, *Yersinia enterocolitica* and *V. cholerae* [115–117]. Furthermore, in a recent study on *A. baumannii*, which does not have PagP, homologues of LpxL and LpxM were shown to promote lipid A acylation to prevent the activities of C18G and polymyxin [118].

In addition to changing surface charge and permeability of the OM, the outermost long polysaccharide chain in LPS, called O-antigen, confers an extra AMP barrier to Gram-negative bacteria [72]. Both LPS core and the O-antigen have been proven essential for AMP resistance in *B. cenocepacia* and *Brucella abortus* using mutants that lack the respective sugar structures in LPS [119,120].

Finally, during symbiotic interaction with insects, changes in the bacterial surface structure are observed that decrease AMP resistance and increase susceptibility to host innate immune defences. Namely, *Burkholderia* species were shown to lose the LPS O-antigen during symbiotic colonization of the gut of the bean bug, *Riptortus pedestris*, resulting in increased susceptibility to purified host AMPs [121]. For a detailed review on insect–symbiont interaction, the reader is referred to the article by Masson *et al*. [122].

## 5. Alteration of the cytoplasmic membrane structure

After passing through all the outer barriers on the bacterial surface, AMPs finally confront the cytoplasmic membrane. Because this is the major target of AMPs, bacteria frequently have strategies to modify the membrane in a fashion that decreases AMP attraction and insertion. The most abundant phospholipids in bacterial membranes are phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG, also called cardiolipin), which have anionic head groups that attract CAMPs. This attraction may be perturbed, for example, by amino-acylation of the PG head group, which masks anionic phosphates with cationic primary amines. Originally described in *S. aureus*, the bifunctional, integral membrane protein, multipeptide resistance factor (MprF) is responsible for amino-acylation of PG with lysine, which results in AMP resistance by electrostatic repulsion [123]. The C-terminal synthase domain of MprF uses PG and aminoacyl–tRNA as substrates. The synthesized aminoacyl-PG is translocated to the outer leaflet of the cytoplasmic membrane by an N-terminal flippase domain in MprF [124]. In a recent study performed in *S. aureus*, further detailed structural information on MprF, the only known bacterial phospholipid flippase, was revealed, including multi-domain interaction and oligomerization [125]. MprF proteins of different bacteria use different amino acid and phospholipid substrates, an adaptation that may explain why MprF is widely spread among bacteria except for enterobacteria [126]. While the majority of bacteria including *Staphylococcus* and *Bacillus* species only generate Lys-PG from PG and lysyl-tRNA, some other bacteria use a different combination of substrates: Lys-PG and Lys-CL in *Listeria monocytogenes* [127], Ala-PG in *P. aeruginosa* [128], Lys-PG and Ala-PG in *Clostridium perfringens* [129]. Compared with other bacteria that are devoid of MprF such as group A or B streptococci [76,130], staphylococci have an especially thick cationic surface barrier, possibly explaining why staphylococci are particularly resistant to CAMPs. Although PG lysylation has been reported mostly in Gram-positive bacteria, there are some studies that report the presence of Lys-PG in Gram-negative bacteria such as *Caulobacter crescentus* [131], *Rhizobium tropici* [132] and *P. aeruginosa*.

In addition to lipid A, the PagP protein also palmitoylates PG on the OM [133]. As PagP is involved in the homeostasis of membrane hydrophobicity, it is certainly imaginable that the protein may also repair AMP-damaged membranes, which would constitute another AMP resistance mechanism [134]. In general, higher membrane rigidity can be achieved by an increase of saturated acyl chains in the membrane, which has been reported to confer elevated resistance to pediocin and nisin in *E. faecalis* and *L. monocytogenes*, respectively [135,136]. In *S. aureus*, enhanced membrane rigidity can be achieved by increased incorporation of the carotenoid staphyloxanthin, which stabilizes acyl chains in the membrane [137]. In contrast, unsaturated fatty acyl chains increase membrane fluidity, which underlies resistance to thrombin-induced platelet microbicidal proteins (tPMP) in *S. aureus* [138]. Generally, the roles of membrane rigidity and fluidity in AMP resistance are still ambiguous and will require enhanced research efforts.

## 6. Efflux pumps

Even when AMPs have already attached to and inserted in the cytoplasmic membrane, bacteria can still remove them using dedicated efflux pumps, which constitute another important AMP resistance component. The resistance/nodulation/cell division (RND) family transporters, AMP efflux pumps that are present in many Gram-negative bacteria, have been studied for some time [139,140]. A typical member of this family is composed of three components: an inner membrane proton/AMP antiporter, an OM transporter and a periplasmic accessory protein that completes and stabilizes the entire efflux complex [141,142]. For example, MtrCDE of *N. gonorrhoeae* and *N. meningitides* is responsible for resistance to LL-37 and protegrin-1 [106,143]. AcrAB-TolC in *K. pneumoniae* is active on polymyxin B as well as human defensins (HNP-1, HBD-1, 2) [144]. Finally, *V. cholerae* VexAB-TolC protects from polymyxin B [145]. Importantly, some RND family efflux pumps from other bacteria have no function in AMP resistance. In those cases (e.g. AcrAB-TolC of *E. coli*, MexAB-OprM of *P. aeruginosa*), no significant differences in the susceptibilities towards cathelicidin, and various human defensins could be observed using overexpression and isogenic efflux pump gene deletion strains [146]. Finally, in another Gram-negative pathogen, *Y. enterocolitica*, RosAB, a major facilitator superfamily (MFS) efflux pump was reported to confer resistance to polymyxin B [147].

Similar to the *S. aureus* MFS family efflux protein NorA [146], most staphylococcal multidrug-resistance transporters are not active on human AMPs. However, the *S. aureus* MFS efflux pump, QacA, has been shown to confer resistance to tPMP [148]. Including in *S. aureus*, a large number of AMP-exporting efflux pumps in Gram-positive bacteria are ATP-binding cassette (ABC) transporters that are designated for the secretion of, or producer immunity towards, newly synthesized AMPs [149]. For example, NisT of *Lactococcus lactis* exports newly synthesized nisin [150] and EpiFEG of *S. epidermidis* protects from epidermin and the structurally similar gallidermin [151]. Notably, these proteins have a limited role in general AMP resistance because of their narrow substrate specificity. However, the other group of two-component ABC transporters, called BceAB type, are active on a wider range of AMPs, including lantibiotics, cyclic AMPs, glycopeptides, cathelicidin and defensins [149]. While most BceAB-type transporters accept as substrates only one or two classes of AMPs (mostly lantibiotics and bacitracin), the *S. aureus* VraFG ABC transporter confers resistance to a variety of AMPs, including nisin, bacitracin, vancomycin, indolicidin,

LL-37 and HBD-3 [149,152,153]. In addition, *S. aureus* has two more BceAB-type ABC transporters, BraDE and VraDE, but their substrates are limited to nisin and bacitracin [154–156]. BceAB-type ABC transporters have been also found in *B. subtilis*, *S. pneumoniae*, *L. monocytogenes* and many other Gram-positive bacteria [149,157–160]. Of note, this type of transporter often appears to be involved with AMP sensing (see §7). Finally, a unique *S. pneumoniae* dual efflux pump of both the MFS and ABC families, MefE/Mel, is inducible by cathelicidin, and confers resistance to LL-37 as well as macrolides [161,162].

## 7. Antimicrobial peptide sensing systems

Although some bacterial AMP resistance mechanisms, such as the modification of lipid II, represent constitutive changes, most are strictly regulated by bacterial sensor/regulators. This is likely to minimize a fluctuation in anionic homeostasis and expression of energy-consuming resistance mechanisms when they are not needed.

The first Gram-positive AMP sensing system was identified in *S. epidermidis* and named antimicrobial peptide sensor (Aps), also known as GraRS or GraRSX [163]. It is unusual in comprising three components: a membrane-bound AMP sensing histidine kinase (ApsS), a DNA-binding response regulator (ApsR) and a third component (ApsX) of yet unknown function, all of which are essential for AMP-dependent gene regulation. The Aps system regulates the expression of genes involved in major AMP resistance mechanisms, such as the *dlt* operon for TA alanylation, the *mprF* gene for PG lysinylation and the *vraFG* ABC transporter. The latter was recently reported to also be involved in AMP-dependent signal transduction [153]. Interestingly, a very short extracellular loop in ApsS interacts with AMPs and determines substrate specificity [152]. Aps-homologous systems are found in multiple Gram-positive pathogens including *S. pneumoniae*, *L. monocytogenes*, *Bacillus anthracis*, *Clostridium difficile* and *Staphylococcus haemolyticus* [163]. Other Gram-positive two-component systems that sense AMPs and regulate resistance mechanisms include BceSR of *B. subtilis* [157], BraSR, VraSR in *S. aureus* [154] and LiaFSR in streptococci [164,165]. Often, they cooperate with corresponding ABC transporters, such as VraFG in the case of Aps [153].

A central regulator for AMP resistance in Gram-negative bacteria is the PhoPQ two-component system [166,167]. PhoQ is a histidine kinase sensor located in the cytoplasmic membrane and PhoP is the corresponding DNA-binding response regulator. PhoPQ and a further PhoPQ-regulated two-component system, PmrAB, work together to regulate most lipid A modifications involved with AMP resistance, which include PagP-mediated palmitoylation, amine-compound addition to phosphates and alteration of the degree of phosphorylation in lipid A [97,102,107,108,113,115,168]. PhoPQ-homologous systems for AMP resistance are found in various Gram-negative bacteria, including *S. typhimurium*, *Y. pestis* and *Shigella flexneri* [169–172]. *Sodalis glossinidius*, an endosymbiont of the tsetse fly, also has the PhoP/PhoQ system, but by loss of PhoP's sensory capacity constitutively expresses genes facilitating resistance to host AMPs during colonization [173].

## 8. Conclusion

Mechanisms of resistance to AMPs are widespread in bacteria. Many of those are based on changes in the physico-chemical properties of surface molecules and the cytoplasmic membrane. These changes commonly confer moderate levels of resistance and are relatively non-specific. Possibly, they are not exclusively involved with AMP resistance. For example, the primary task of many surface molecules is the formation of a biofilm matrix and that of many secreted proteases the acquisition of nutrients, and most AMP efflux pumps also accept other antimicrobial molecules as substrates. For drug development efforts in the field of AMPs, it is important that AMP resistance, which may develop due to selective pressure [174], is thus not based on dedicated resistance genes that are conferred, such as in the case of many antibiotic resistance mechanisms, by horizontal gene transfer [175]. Rather, these observations mean that for any AMP-based drug, a certain increase of resistance after exposure to the drug is to be expected ('MIC creep'), such as observed for the AMP-related antibiotic, daptomycin [126,176].

Even if many details about bacterial resistance to AMPs have been discovered, several topics await more in-depth investigation, such as membrane repair mechanisms, the role of membrane fluidity/rigidity, the involvement of host components and resistance mechanisms of the Gram-negative inner membrane.

Competing interests. We have no competing interests.

Funding. The authors are supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, US National Institutes of Health.

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