Evolutionary constraints on the acquisition of antimicrobial peptide resistance in bacterial pathogens

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Abstract

Antimicrobial peptides (AMPs) offer a potential solution to the antibiotic resistance crisis. Recent studies have revealed important evolutionary constraints on the evolution and horizontal spread of AMP resistance in bacteria. Here, we summarize these advances and highlight their importance for therapeutic development of AMPs.

Keywords

Antimicrobial peptide resistance, bacterial evolution, antibiotic resistance

Antimicrobial peptides and bacterial resistance

Antimicrobial peptides (AMPs) are multifunctional molecules found among all classes of life. In metazoans, AMPs are key components of innate immunity, and have diverse functions, such as providing the first line of defense against invading pathogens, immunomodulation and regulating the composition of the gut microbiome [1]. The remarkable antimicrobial activity of AMPs has stimulated interest in their therapeutic use against multidrug-resistant (MDR) pathogens.

Although AMPs exhibit potent antibacterial activity, bacteria can evolve genetically-encoded AMP resistance, either as a result of chromosomal mutations [2,3] or by horizontal acquisition of resistance genes, such as mobile colistin resistance (*mcr-1*) [4]. The key defining feature of genetically encoded resistance is that it allows pathogen populations to grow at higher AMP doses. Since AMPs are key effectors of host immunity, resistance to AMPs is likely to increase bacterial virulence by increasing pathogen colonization and evasion of host innate immunity (Figure 1). Also, the development of AMP resistance via membrane modification can influence bacterial susceptibility to commonly used antibiotics (Figure 1). Therefore, understanding the evolutionary principles underlying AMP resistance in pathogens is of paramount importance to develop effective AMP-based therapies.

From an evolutionary perspective, an important question is: why is resistance evolution against host immunity AMPs not widespread in several pathogens? Early studies claimed that it is because of unique properties of AMPs, such as pharmacodynamics [5]. Here, we describe recent advances suggesting that in addition to these properties, important evolutionary constraints, such as fitness trade-offs, functional compatibility and association of AMP resistance genes with mobile genetic elements (MGEs), limit the acquisition of AMP resistance.

De novo evolution of AMP resistance

Physiochemical and mechanistic properties of AMPs influence their interactions with bacteria and shape resistance mechanisms [2,6]. Certainly, AMPs show diverse mechanisms of action, including fibrils and nanonets formation, targeting cell membrane components (such as lipopolysaccharide, phosphatidylethanolamine, lipoteichoic acid and the peptidoglycan precursor lipid II) and/or intracellular processes (DNA replication, protein synthesis, etc.) [1,7]. One of the key insights from laboratory evolution experiments has been that AMP

resistance often evolves through membrane modification, resulting in reduced affinity for AMPs [2]. At the molecular level, some of the AMP resistance mechanisms are comparable to antibiotic resistance mechanisms (Figure 2). For example, enzymatic inactivation of AMPs by proteases is analogous to the destruction of β-lactam antibiotics by β-lactamases, and membrane modifications that reduce binding of AMPs, are analogous to the exchange of D-alanine with D-lactate that decreases vancomycin's binding to the bacterial cell wall. Similarly, heteroresistance which is an important phenomenon across several antibiotics, has also been linked with bacterial resistance to colistin (via altered expression of PmrAB and PhoPQ) [8] (Figure 2).

Altogether these examples suggest some superficial similarities in the mechanisms of resistance to antibiotics and AMPs. However, the evolutionary responses to these two classes of antimicrobial are clearly different, as *de novo* resistance to AMPs evolves at a much lower rate than antibiotics, with the notable exception of polymyxin B which is not a host defense peptide [2]. In part, this difference is likely to reflect differences in the modes of action of AMPs and antibiotics. Previous *in vitro* studies have argued that AMP resistance evolves at a low rate due to AMP-associated properties, such as pharmacodynamics [5] and targeting the cytoplasmic membrane, which is thought to be a bacterial 'Achilles heel' that cannot rapidly evolve.

Whilst these properties are clearly important, recent work has revealed additional constraints on the evolution of AMP resistance (Figure 2). Fitness costs play a key role in limiting the spread of antibiotic resistance in pathogens. Acquisition of AMP resistance reduces the growth rate and competitive ability of bacteria [9]. However, compensatory adaptation can offset this cost of AMP resistance. Second, AMP resistance is often associated with pleiotropic trade-offs, such as collateral sensitivity between functionally dissimilar AMPs [6]. This suggests that reciprocal collateral sensitivity to cocktails of co-expressed AMPs may constrain the evolution of AMP resistance. This, in turn, might explain why eukaryotes produce a large number of diverse AMPs [5].

Moving forward, an important challenge is to understand additional barriers to the acquisition of AMP resistance *in vivo*. For example, do pathogens require multiple mutations to evolve clinically relevant levels of resistance to AMPs [3], and whether multiple mutations reduce *in vivo* fitness and induce deleterious side effects in a complex host environment. Moreover, studies argue that in higher organisms, synergistic interactions between AMPs

increase bacterial killing [5]. However, it should be noted that despite the increased killing, synergistic combinations may favour the evolution of resistance, as seen in the case of antibiotics [10]. This counter-intuitive effect arises because mutations that confer resistance to one drug in a synergistic pair not only provide a large fitness advantage but also eliminate the added synergistic effect that emerges from the effect of the two drugs on sensitive cells [10]. Future studies should explore whether synergistic or antagonistic AMP pairs could forestall the emergence of AMP resistance in pathogens.

Horizontal acquisition of AMP resistance

Horizontal gene transfer (HGT) mediated by plasmids, phages and transposons, plays an important role in the dissemination of antibiotic resistance among bacterial pathogens. In sharp contrast, we have a limited understanding of horizontal spread of AMP resistance.

Resistance to AMPs is probably prevalent in some complex microbial communities, such as human gut microbiome [11,12]. Gut commensals are regularly exposed to intestinal AMPs, and have evolved intrinsic resistance against them [11]. For example, Bacteroidetes resist inflammation-associated AMPs by *lpxF*-mediated dephosphorylation of lipid A [11]. Interestingly, MCR-1 appears to have been acquired from pig commensal bacteria, most likely *Moraxella* [13], where it may provide resistance to host AMPs. These observations suggest that the host-microbiome may be an important source of transferable AMP resistance genes.

Notably, AMP resistance genes are transferred at a lower rate than antibiotic resistance genes in the gut microbiome [12]. And this difference could stem from differences in the functions between AMP resistance and antibiotic resistance genes [12]. Known AMP resistance genes mainly function by modifying complex cellular structures that are formed by networks of interacting proteins, such as bacterial cell membrane [2,6]. As such, the phenotype of these genes (i.e., AMP resistance) and its associated fitness costs, are likely to be highly contingent on the host genetic background, constraining the ability for AMP resistance to be horizontally transferred [12]. Mobile antibiotic resistance genes, on the other hand, usually function as ‘stand-alone’ enzymes that modify antibiotics or their cellular targets, ensuring their transfer, even between distantly related bacteria. This functional compatibility hypothesis makes several interesting predictions – for example, proteases that degrade AMPs may be more mobile than membrane-modifying AMP resistance genes.

It is also possible that AMP resistance genes are transferred at a low rate because they lack associations with MGEs. Theory predicts that genes that are regularly subject to positive selection will become integrated into immobile regions of the genome [14]. Given the importance of AMPs in innate immunity, it is likely that commensal bacteria are routinely subjected to AMP selection. Consistent with this theory, a significantly smaller fraction of AMP resistance genes are closely linked to known plasmids and integrative conjugative elements in the human gut microbiome compared to antibiotic resistance genes [12]. Antibiotics, on the other hand, are mainly derived from soil bacteria and fungi, suggesting that soil bacteria are routinely selected for antibiotic resistance. Interestingly, soil antibiotic resistance genes are also weakly associated with MGEs [15].

Moreover, MCR-1 highlights the importance of association with MGEs in AMP resistance and has provided a real-time window into this process of AMP gene mobilization and immobilization. Antibiotic resistance genes are thought to be mobilized by the capture of chromosomal resistance genes by transposons, followed by the movement of transposons to conjugative plasmids, followed by the horizontal transfer of plasmids between bacterial hosts. Colistin (polymyxin E) has been used at a large-scale in agriculture since the 1980s, but the mobile *ISApl1* transposon that contains the *mcr-1* was only formed sometime in the mid-2000s [13]. Following the initial formation of *ISApl1-mcr-1-orf-ISApl1* transposon, the MCR-1 transposon rapidly spread to multiple plasmids (mostly narrow-host-range plasmids IncI2 and IncX4) that were transferred between *E. coli* strains [13]. However, this gene has become rapidly immobilized because of chromosomal integration, degeneration of the *ISApl1* transposon and loss of conjugative genes in MCR-1 plasmids [13].

Concluding remarks and future perspectives

Recent studies using high-throughput evolutionary experiments and functional genomics have revealed key evolutionary constraints on AMP resistance. Although in pathogen populations, these constraints limit the evolution of resistance to host AMPs, it is unclear to what extent these constraints will be able to stop the spread of resistance to AMPs that used therapeutically. An interesting avenue for future work will be to explore evolutionary barriers to AMP resistance in clinically relevant pathogens, and identify strategies that exploit these constraints, such as using AMP cocktails to minimize the efficacy of selection for resistance or using adjuvants to maximize the costs of AMP resistance. The example of colistin resistance

clearly highlights the need to evaluate the potential for resistance evolution as part of the pre-clinical development of therapeutic AMPs, including evaluating the potential for AMP resistance to be acquired by HGT from human and animal microbiomes.

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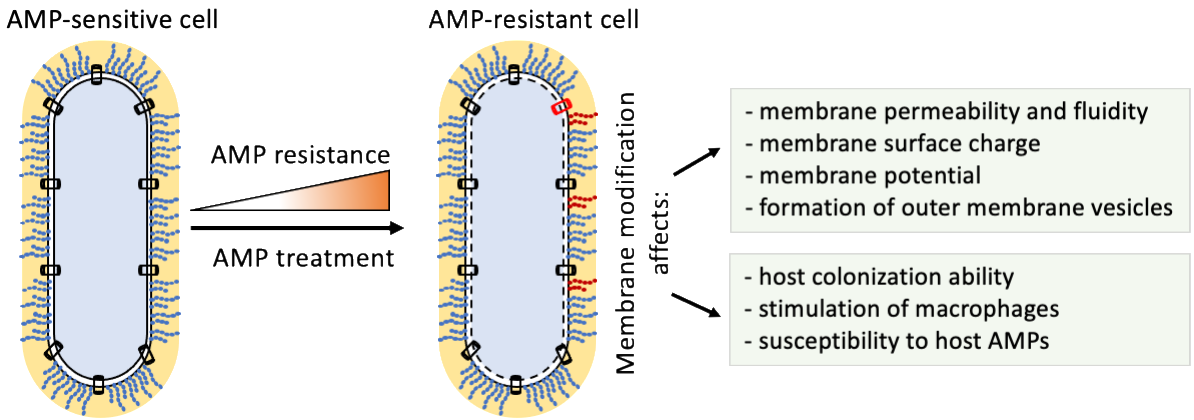

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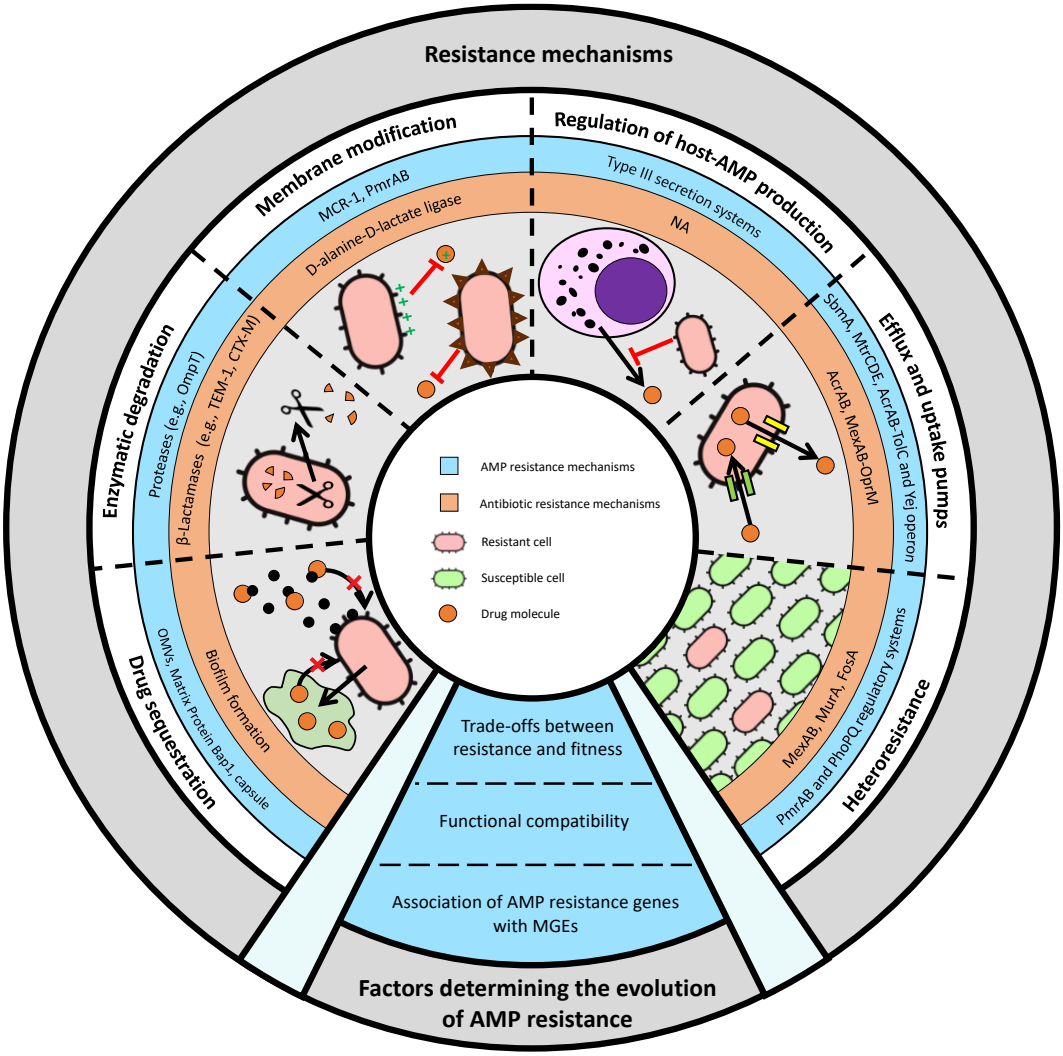
187 **Figure 1. Pleiotropic effects of membrane modification on bacterial virulence and**
188 **susceptibility to antibiotics.** Bacterial resistance to AMPs often involves modification of
189 membrane components, such as lipopolysaccharide (LPS) and phospholipids. Mutations in
190 these components alter membrane properties (e.g., permeability, fluidity and surface charge)
191 that, in turn, can affect bacterial susceptibility to antibiotics. On the other side, membrane
192 components act as modulators of host immune response and thus alter bacterial virulence.



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194 **Figure 2. Different mechanisms of resistance to AMPs and antibiotics, and the factors that**
195 **shape the evolution of AMP resistance in bacteria**. OMVs – outer membrane vesicles, NA -
196 no data available