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Review

Mechanisms of resistance to antimicrobial peptides in staphylococci☆

Hwang-Soo Joo, Michael Otto *

Pathogen Molecular Genetics Section, Laboratory of Human Bacterial Pathogenesis, National Institute of Allergy and Infectious Diseases (NIAID), U.S. National Institutes of Health (NIH), Bethesda, MD, USA

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ABSTRACT

Staphylococci are commensal bacteria living on the epithelial surfaces of humans and other mammals. Many staphylococci, including the dangerous pathogen *Staphylococcus aureus*, can cause severe disease when they breach the epithelial barrier. Both during their commensal life and during infection, staphylococci need to evade mechanisms of innate host defense, of which antimicrobial peptides (AMPs) play a key role in particular on the skin. Mechanisms that staphylococci have developed to evade the bactericidal activity of AMPs are manifold, comprising repulsion of AMPs via alteration of cell wall and membrane surface charges, proteolytic inactivation, sequestration, and secretion. Furthermore, many staphylococci form biofilms, which represents an additional way of protection from antimicrobial agents, including AMPs. Finally, staphylococci can sense the presence of AMPs by sensor/regulator systems that control many of those resistance mechanisms. This article is part of a Special Issue entitled: Bacterial Resistance to Antimicrobial Peptides.

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Contents

1. Introduction

Staphylococci are a major cause of infections in both health care and community settings [1]. Antibiotic resistance is widespread in staphylococci, significantly complicating treatment. Methicillin-resistant *Staphylococcus aureus* (MRSA) in particular has been estimated to cause nearly 20,000 deaths every year in the Unites States, which is more than reported for HIV/AIDS [2].

Staphylococcal infections mostly originate from colonizing strains. *S. aureus* and the coagulase-negative *Staphylococcus epidermidis* are the most common commensal bacteria colonizing the human nose and skin [3,4]. Approximately 30% of the population carry *S. aureus* and 20% are persistent carriers [5,6]. Importantly, it has been demonstrated that colonization with *S. aureus* poses a risk for subsequent

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* Corresponding author at: 9000 Rockville Pike, Building 33 1W10A, Bethesda, MD 20892, USA. Tel.: +1 301 443 5209; fax: +1 301 480 3632.
E-mail address: motto@niaid.nih.gov (M. Otto).

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infection [7]. When the protective layer of the human epithelium is breached and mechanisms of host immunity fail, staphylococcal infections such as bacteremia or pneumonia can become extremely dangerous and life-threatening [8].

The innate immune system plays a major role in fighting off staphylococcal infections. Antimicrobial peptides (AMPs) represent the first line of innate immune defenses on the human skin and also form part of the mechanisms by which bacteria are eliminated in the neutrophil phagosome after phagocytosis. Many different organisms, including humans, produce AMPs; and many human AMPs have been discovered that are active against staphylococci. AMPs in humans belong to two major groups: defensins and cathelicidins. All of these have a positive net charge and are therefore collectively called cationic antimicrobial peptides (CAMPs). There is one exception in humans with a negative net charge, namely dermcidin, an anionic AMP originally isolated from human sweat [9].

As human AMPs have evolved to play a pivotal role in innate immunity, staphylococci as human colonizers have developed versatile strategies to evade AMP activity during both colonization and infection [10]. This includes, for example, surface charge alteration, extracellular proteases, exopolymers, and efflux pump proteins, mechanisms that are regulated by specific sensor/regulator systems (see Table 1). This review will give an overview on staphylococcal mechanisms of AMP sensing and strategies of AMP resistance.

2. Staphylococcal sensing of antimicrobial peptides

Staphylococci have a three-component antimicrobial peptide sensor (*aps*) system, which was the first Gram-positive bacterial AMP sensing system to be discovered by studies on *S. epidermidis* [11]. It is composed of a classical two-component system with a sensor histidine kinase (ApsS) and a DNA-binding response regulator (ApsR) in addition to a third component (ApsX), which appears only in staphylococci and whose exact function is still unclear [11]. ApsRS is also known as GraRS, based on earlier studies, in which this two-component system was described to provide resistance against glycopeptide antibiotics [12,13]. ApsS is a membrane protein with an AMP-sensing extracellular loop consisting of 9 amino acids with negative net charge [11]. Direct interaction of that loop with AMPs was shown in the original publication on the *S. epidermidis* Aps system with specific antibodies that blocked induction, and was further confirmed more recently in *S. aureus* [14]. The *S. aureus* Aps system appears to be more limited regarding the spectrum of AMPs to which it reacts, whereas *S. epidermidis* responds to a larger variety of peptides. For example, the Aps systems in both species recognize LL-37 and indolicidin, while only the *S. epidermidis* system recognizes the important AMP human beta-defensin 3 (HBD-3), which provides anti-staphylococcal activity on human skin. Using genetically engineered strains expressing hybrid ApsS proteins, these differences in AMP inducibility between the *S. aureus* and *S. epidermidis* Aps systems have been shown to be due to the amino acid sequence difference within the short loop region of ApsS [15]. AMP selectivity of the *S. aureus* Aps system was also further studied in MRSA strains [16]. Clearly, the intriguing nature of the AMP selectivity of ApsS still needs more investigation, in particular regarding its biological significance.

There have been multiple studies in *S. aureus* attempting to elucidate the mechanism of the Aps sensing/regulation system in more detail. Although the precise function of ApsX is yet to be determined, genome/transcriptome analyses and protein–protein interaction studies have revealed that it plays a key role in signal transduction, connecting the two parts of a sensor/regulator complex comprised of the VraFG ABC transporter, a target of Aps-dependent regulation, in addition to ApsRSX itself [17,18]. In particular, it could be demonstrated that the expression of *apsRS* and the sensing of AMPs by Aps appear to be dependent on VraFG [16,18]. Thus, according to those recent studies, VraFG may play a more active role in the Aps sensing/regulation system than previously expected.

While many genes appear to be regulated by the Aps system based on the analysis of gene deletion strains, induction experiments with AMPs revealed what appears to be the most important feature of Aps-dependent gene regulation, namely that the Aps system up-regulates expression of genes encoding major AMP resistance mechanisms in staphylococci [15]: AMP-activated Aps induces expression of (i) the *dlt* operon that incorporates D-alanine into teichoic acids, which contributes to neutralizing the negative net charge of the staphylococcal cell wall, (ii) the *mprF* gene that forms lysyl-phosphatidylglycerol (Lys-PG), which reduces the negative net charge of the cellular membrane, and (iii) the *vraFG* ABC transporter genes (Fig. 1). Increased expression of the Dlt and MprF systems confer resistance to CAMPs by altering the cell surface charge, as discussed further below, while VraFG has been proposed to be involved in AMP export, a notion based on the fact that a *vraFG* deletion mutant showed decreased resistance to several AMPs [15]. However, the more recent findings indicating a role for VraFG as part of the Aps/VraFG sensing complex suggest that this may only be a secondary activity of VraFG.

As the Aps system governs the expression of the main AMP resistance toolbox in staphylococci, it is considered a pivotal regulatory system of staphylococcal resistance to AMPs. The importance of Aps for bacterial survival is reflected by the finding that it significantly impacts resistance to killing by human neutrophils, which use AMPs as one of two key mechanisms to eliminate bacteria after phagocytosis (the other being reactive oxygen species), based on experimental data from both *S. aureus* and *S. epidermidis* [19]. Furthermore, an *apsS* deletion mutant of strain *S. aureus* MW2 showed a significantly lower bacterial burden in kidneys in a murine infection model, indicating an important role of AMP sensing during *S. aureus* infection [15]. However, other staphylococcal regulatory systems, such as the global regulators Agr, SarA and SaeRS, also regulate AMP resistance, mainly by controlling expression of secreted proteases with low substrate specificities that degrade AMPs [20]. For example, the *S. epidermidis* exoprotease SepA (a homologue of *S. aureus* aureolysin) significantly contributes to the evasion of killing by human neutrophils [19]. The activation of proteolytic defense mechanisms via Agr, SarA, and SaeRS can be stimulated by AMPs regardless of their charge and likely is a result of a general disturbance of membrane function and thus resembles a general stress response [20]. Finally, it is also noted that the Aps system has been reported to be involved with providing resistance to environmental stresses such as high temperature or oxidative stress [17].

Recently, there have also been reports on AMP resistance-related novel regulatory systems in staphylococci, with or without a relation

Table 1
Staphylococcal resistance mechanisms that target AMPs.

Resistance mechanism	Gene	Target AMPs
AMP sensing	*apsSRX*	Most cationic AMPs with some selectivity for *S. aureus* [11,15]
	vraFG (+ *apsSRX*)	Colistin, polymyxin B, HNP1, RP-1 [16,18]
	braSR/braDE/vraDE	Bacitracin, nisin [96]
PG lysylation	*mprF*	Most cationic AMPs [23]
TA alanylation	*dltABCD*	Most cationic AMPs [44]
Exopolymers	*icaADBC* (PIA)	HBD3, LL-37, DCD-1 [68]
	capBCAD (PGA)	HBD3, LL-37, DCD-1 [67]
Extracellular proteases	*aur/sepA*	LL-37 [73,75]
	sspA/esp	LL-37[a][72]
Staphylokinase	*sak*	HNP1, HNP2, LL-37[b][81,84]
ABC transporters	*vraFG*	Vancomycin, polymyxin B, colistin [12,18]
	braSR/braDE/vraDE	Bacitracin, nisin [96]

[a] Degraded but still active.
[b] Bound to activate fibrinolysis.