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Review

Mechanisms of resistance to antimicrobial peptides in staphylococci☆

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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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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.

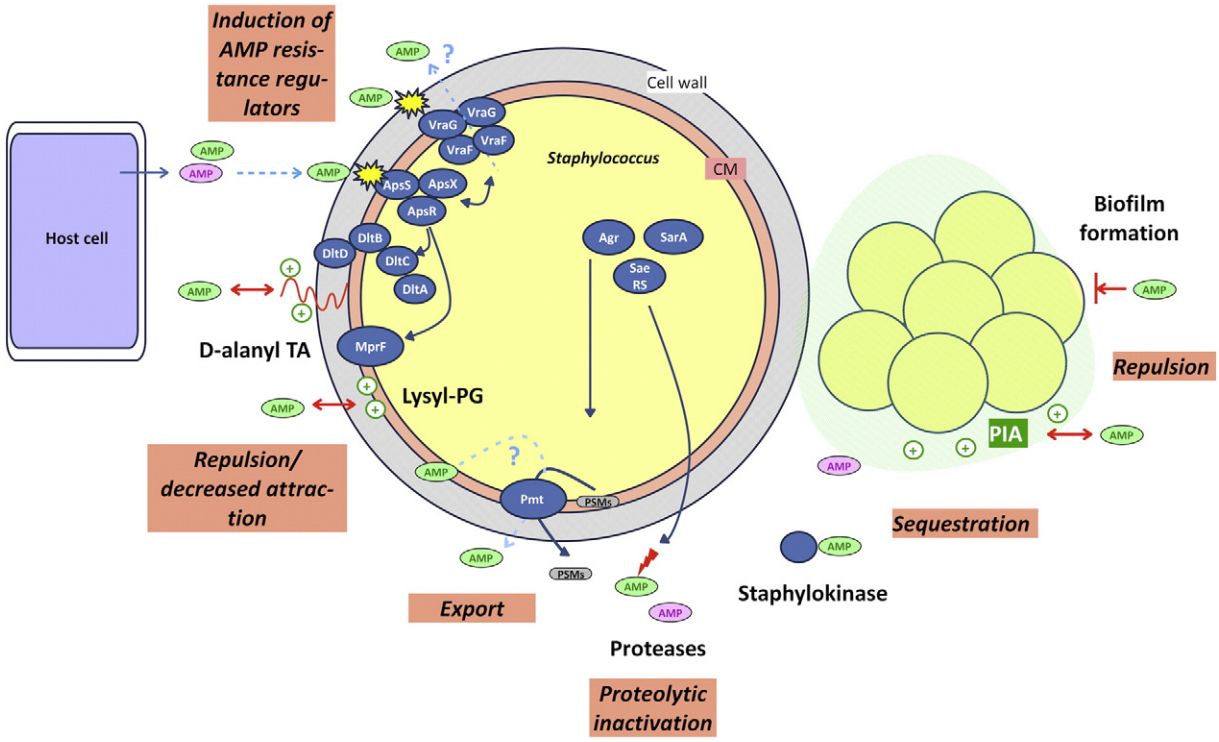

Fig. 1. Overview over AMP resistance mechanisms in staphylococci. Host cells produce CAMPs (positively charged, in green) or, rarely, anionic, negatively charged AMPs such as dermcidin (in red). CAMPs induce the Aps/VraFG regulatory system, leading to increased expression of the Dlt system, which D-alanylates TA, and MprF, which modifies PG with lysyl residues. Both mechanisms contribute to repulsion, or decreased attraction, of CAMPs. Aps/VraFG also induces the expression of VraFG, which itself may be involved in AMP export. Export pumps such as the Pmt PSM ABC transporter expel membrane-active AMPs such as the staphylococcal PSM toxins, but also possibly host-produced AMPs, from the cytoplasmic membrane (CM). Secreted proteases with commonly low substrate specificity degrade AMPs (both anionic AMPs and CAMPs) and are regulated by global regulators such as Agr, SarA, and SaeRS. Staphylokinase inactivates CAMPs by sequestration, a mechanism also seen with AMPs and oppositely charged surface polymers (sequestration of an anionic AMP by the cationic exopolysaccharide PIA is shown as example). The formation of biofilms contributes to AMP resistance by a multitude of mechanisms that include decreased penetration. In addition, the cationic biofilm polymer PIA may repel CAMPs.

to Aps. For instance, Stk1, a serine/threonine kinase, which has previously been known as a methicillin resistance factor in MRSA, was reported to phosphorylate ApsR, thereby impacting the expression of the Aps regulon [21]. Furthermore, in another recent study a transmembrane potential ($\Delta\psi$) sensor/regulator, the two-component system LytSR, was described to control resistance to AMPs in a manner that is unrelated to Aps-dependent gene regulation [22].

3. Staphylococcal strategies to evade killing by antimicrobial peptides

3.1. Aminoacylation of phosphatidylglycerol by MprF

The phospholipids phosphatidylglycerol (PG) and cardiolipin are major lipid components of the bacterial cytoplasmic membrane; as they are both anionic, the membrane has a negative surface charge, attracting cationic AMPs. Staphylococci and several other bacteria have a gene called *mprF* (for multiple peptide resistance factor), whose protein product is responsible for the aminoacylation of PG via ester bond formation between the glycerol moiety of PG and the carboxyl group of lysine or alanine, which results in a reduction of the negative charge of the bacterial membrane, diminishing attraction of CAMPs [23,24]. While MprF in staphylococci only produces lysyl-PG (Lys-PG), other bacteria generate alanyl-PG (Ala-PG) or both Lys-PG and Ala-PG via the enzymatic activity of MprF [24,25].

MprF is a membrane protein consisting of 14 transmembrane domains (TMDs). The C-terminal 6 TMDs of MprF produce Lys-PG by recruiting PG and lysyl-tRNA as substrates. The N-terminal 8 TMDs translocate Lys-PG from the inner leaflet to the outer leaflet, an activity representing the first described bacterial phospholipid flippase [26]. Both domains are required for AMP resistance. By heterologous co-expression of an Ala-PG synthase domain and a Lys-PG flippase domain, the flippase domain has recently been shown to be responsible for the substrate specificity of MprF [27]. Importantly, it has been reported that point mutations in a specific region of *mprF* lead to resistance toward daptomycin, a lipopeptide antibiotic of last-resort for multi-drug resistant bacteria [28]; and multiple studies have demonstrated a linkage between point mutations in *mprF* and daptomycin resistance in clinical isolates and laboratory strains [29–32]. In a recent comparative study on daptomycin-susceptible and -resistant MRSA isolates, a specific point mutation in *mprF* was shown to be important for gaining daptomycin resistance based on an enhanced MprF phenotype [33]. Finally, interesting details on the aminoacyl-PG hydrolase as a counterpart of MprF in the aminoacyl-PG homeostasis were recently reported by two independent studies, one performed in *Enterococcus faecium* [34] and the other in *Pseudomonas aeruginosa* [35]. Newly found hydrolases in both bacterial strains degrade aminoacyl-PG back to PG to maintain the aminoacylation level of the lipid membrane, which also appears to be involved in additional functions such as antimicrobial resistance and cell growth. The findings that were reported in those two studies provide new aspects that help to understand how aminoacyl-PG homeostasis is achieved with one or multiple aminoacyl-PG species, and how this affects resistance to antimicrobial compounds. Although the corresponding enzyme has yet to be discovered in staphylococci, aminoacyl-PG homeostasis by hydrolase activity requires to be studied together with aminoacyl-PG synthases such as

MprF for a better understanding of mechanisms of staphylococcal resistance to AMPs.

3.2. Teichoic acids and the role of D-alanylation of teichoic acids by the products of the dlt operon

Staphylococci and other Gram-positive bacteria have teichoic acids (TAs) in their cell wall [36]. TAs are a variety of anionic polymers of phosphodiester-linked polyol phosphate repeating units with disaccharide anchoring units [37,38]. The repeating units are composed of one or both types of glycerol phosphate (Gro-P) and ribitol phosphate (Rbo-P) with frequent modification by D-alanine and/or mono-/di-saccharides, a mechanism that provides zwitterionic property. According to the anchoring location, TAs are further classified into two groups, wall teichoic acid (WTA) and lipoteichoic acid (LTA). WTA is covalently linked to the cell wall via a phosphodiester bond between an N-acetyl glucosamine (GlcNAc) in WTA and an N-acetyl muramic acid (MurNAc) moiety of peptidoglycan, whereas the disaccharide of LTA is attached to the glycerol moiety of diacylglycerol in the outer leaflet of the cell membrane. In *S. aureus*, WTA consists of 2 Gro-Ps and 40 Rbo-Ps, while LTA comprises 18 to 50 Gro-Ps only [39]. Readers are referred to dedicated review articles for expansive reviews about WTA [40] and LTA [41,42], a concise review about *S. aureus* TA [39], and a comprehensive review about TA from a physiological viewpoint [43].

Staphylococci incorporate D-alanine into the glycerol moiety of repeating units in both WTA and LTA. As this happens via esterification of the D-alanine carboxy group and leaves the positively charged free amine, the reaction results in TA with a decreased overall negative net charge, lowering CAMP attraction [44], in a manner that is similar to that by which MprF incorporates L-lysine into PG and thereby diminishes the negative charge of the membrane surface. The *dltABCD* operon is responsible for this D-alanylation mechanism and thus represents an important genetic locus conferring resistance to CAMPs in staphylococci and other Gram-positive bacteria. DltA activates D-alanine with ATP and transfers it to DltC, a D-alanyl carrier protein [45,46]. A transmembrane protein, DltB, and a membrane-anchored putative esterase/thioesterase, DltD, are believed to complete the D-alanylation of LTA, but the exact mechanistic activities of these two proteins are still unclear [47,48]. It is also assumed that the D-alanyl moiety can be transferred from LTA to WTA [49]. As previously mentioned, expression of the *dlt* operon is controlled by the Aps system [11,15] and, additionally, by cations [50].

In addition to their classical role as a defensive barrier in Gram-positive bacteria, teichoic acids also play various other biological roles in staphylococci. For example, WTA is involved in the regulation of peptidoglycan biosynthesis and cell division by binding to penicillin-binding proteins (PBPs) or autolysins [51–53]. Interestingly, in contrast to previously observed resistance mechanisms targeted at other CAMPs, WTA deficiency achieved by deletion of *tagO* in *S. aureus* has been shown to lead to increased resistance selectively to HBD3 and group IIA phospholipase A(2), indicating multifactorial properties of WTA-dependent AMP resistance [54]. WTA is currently drawing even more attention owing to the important roles it has recently been reported to play in horizontal gene transfer among bacterial pathogens [55] as well as nasal colonization of *S. aureus* through the WTA receptor, SREC-I [56,57].

3.3. Exopolymers and biofilms

Polysaccharide intercellular adhesin (PIA, also known as PNAG, poly-N-acetylglucosamine) is a cationic exopolysaccharide produced by the *icaADBC* locus in *S. aureus*, *S. epidermidis*, and other staphylococci [58–61]. Homologous systems are present in a variety of other bacteria [62,63]. PIA/PNAG is a linear homopolymer of partially de-acetylated GlcNAc. Importantly, de-acetylation by IcaB results in a positive net charge of PIA/PNAG, which is crucial for resistance to AMPs [64]. In *S. aureus* the *ica* locus and thus expression of PIA/PNAG is subject to phase variation by a frame shift mutation within *icaC* likely occurring via slipped-strand mispairing [65]. Another important bacterial exopolymer, poly-γ-glutamic acid (PGA) is produced by coagulase-negative staphylococci and some other species, but not *S. aureus* [66,67]. The *capBCAD* operon is responsible for the production of PGA. Interestingly, PIA/PNAG and PGA provide protection to both cationic and anionic human AMPs such as LL-37, HBD3, and dermcidin [64,67,68], although those AMPs have opposite net charges. This indicates that resistance mechanisms to AMPs facilitated by these two exopolymers do not only include simple electrostatic repulsion but also mechanisms based on electrostatic sequestration or charge-independent mechanical barrier functions. In addition, both molecules contribute to the evasion from neutrophil phagocytosis [67,68]. More recently, another type of PIA/PNAG with partial sulfation has been discovered in *S. epidermidis*, whose biological role and biosynthetic pathway still need to be elucidated [69].

PIA/PNAG and PGA both impact pathogen success in in-vivo models of staphylococcal biofilm-associated infection on indwelling medical devices, but only PIA/PNAG has been shown to influence biofilm formation in vitro [63,67]. PGA, however, is likely important for biofilm-specific AMP resistance according to a genome-wide analysis of gene expression that has shown up-regulation of the *cap* locus in *S. epidermidis* biofilms [70]. Of note, because in addition to AMP resistance, PIA/PNAG plays a crucial role in in-vitro biofilm formation and in-vivo biofilm-associated infection [61,64,71], it contributes to AMP resistance by two mechanisms — as a surface exopolymer that repels CAMPs and as a component of the biofilm matrix, a double mechanism likely shared by other staphylococcal exopolymers.

3.4. Extracellular proteins

Staphylococci produce a series of proteases, such as *S. aureus* V8 protease [72] or aureolysin [73], some of which are known to degrade human AMPs. Aureolysin is a zinc-dependent metalloprotease with low substrate specificity [74]. Its homologue in *S. epidermidis* is SepA, which is encoded by the *sepA* gene [75]. The aureolysin-type proteases are well known to proteolytically inactivate LL-37, the only cathelicidin found in humans. In contrast, the serine protease V8 protease, encoded by the gene *sspA*, can degrade LL-37, but the resulting fragment was reported to be still active against *S. aureus* [73]. Furthermore, we already mentioned above that *S. epidermidis* SepA contributes to evading neutrophil killing, likely by inactivating AMPs and other protein-dependent bactericidal mechanisms in the neutrophil phagosome [19]. Of note, staphylococcal serine proteases, including V8 and *S. epidermidis* Esp, also play important roles in the evasion of human immune defenses conferred by mechanisms other than AMPs, by degrading, for example, α1-proteinase inhibitor, complement component 5, or fibrinogen [76,77]. Finally, the expression of these proteases is mostly up-regulated by *agr* and repressed by *sarA* [78–80], resulting in significant effects that these regulatory systems have on AMP resistance.

S. aureus also secretes staphylokinase, which sequesters α-defensins (HNP-1 and 2) [81,82]. For decades, activation of plasminogen has been the only known function of staphylokinase [83] until multiple binding sites for HNPs, which are distinct from the plasminogen binding site, were revealed in staphylokinase [81]. The bactericidal activities of α-defensins in complex with staphylokinase are significantly lower than without staphylokinase, an effect that is independent of plasminogen. In addition, staphylokinase binds to LL-37 to increase plasminogen activation and fibrinolysis [84]. Interestingly, streptokinase (Ska) found in streptococci exploits Ska-activated host plasmin to degrade LL-37 cooperatively [85].

Furthermore, staphylococcal extracellular enzymes also play a crucial role in the escape from neutrophil extracellular traps (NETs). Some neutrophils release DNA followed by decoration with histone

and granule proteins to build NETs [86]. This process, also called NETosis, helps immune cells to protect themselves from bacterial invasion by trapping and killing exogenous microbes. A recent study revealed a new strategy of *S. aureus* to escape from NETs, namely that extracellular nuclease and adenosine synthase A degrade and synthesize 2′-deoxyadenosine to facilitate macrophage apoptosis [87].

3.5. ABC transporters

In many organisms, multi-drug exporters with broad substrate specificity provide resistance to a variety of antimicrobial compounds [88]. Although many transporters have been discovered in staphylococci that confer resistance to antibiotics [89], most staphylococcal multidrug exporters are not active against human AMPs. For example, one of the most important staphylococcal multidrug efflux pumps, NorA, does not protect from AMPs such as human defensins and LL-37 [90]. Nevertheless, some ABC transporters that provide resistance from AMPs have been described staphylococci, but most of them have narrow substrate specificity and predominantly confer resistance to lantibiotics, which are post-translationally modified peptide bacteriocins containing the amino acid lanthionine [91]. These lantibiotic exporters are involved in secretion or producer immunity, as many lantibiotics such as epidermin, gallidermin, Pep5, or aureodermin (Bsa) are produced by staphylococci themselves [92]. According to Gebhard's classification of AMP transporters, a large number of AMP transporters known in staphylococci are classified into lantibiotic-associated groups, such as the SunT/NisT group whose members are involved with the secretion of newly synthesized AMPs, or the LanFEG group members that are important for producer self-protection from lantibiotic activity [93–95]. In contrast to those groups, the transporters of the BceAB group are more broadly involved in AMP resistance due to less narrow substrate specificities, expelling at least two different types of AMPs, bacitracin, which is a cyclic AMP, and the lantibiotic nisin. In addition to transcriptional regulation by BraSR (also known as BceSR or NsaSR), BraDE (also known as BceAB or NsaAB) and VraDE have been shown to be involved in bacitracin/nisin sensing and detoxification, respectively [96–98]. While BraDE and VraDE accept only those selected bacteriocins as substrates, the VraFG transporter, which also belongs to the BceAB group, protects from indolicidin, vancomycin, LL-37 and HBD3 [15]. However, as mentioned above, there is the caveat that it needs to be confirmed that this protection is due to a transport function of VraFG rather than its participation in the Aps sensing/regulation system [18,96]. In fact, the presence of adjacent two component sensor/regulator systems (e.g. BceSR) on the genome and the involvement of the transporters in AMP sensing mechanism are general features of BceAB-type transporters [99,100]. Notably, BceAB-type sensing transporters often share their sensing information with other transporters or sensor/regulator systems.

The primary biological features of the staphylococcal phenol-soluble modulin (PSM) peptides are believed to be their cytolytic, pro-inflammatory, and biofilm-structuring activities [101]. However, PSMs also exhibit antimicrobial effects that are assumed to be a side effect of the membrane-damaging capacities of those peptides that are predominantly targeted at eukaryotic membranes [102]. Our group has recently identified an ABC transporter, named Pmt (phenol-soluble modulin transporter), as the transporter that secretes PSMs and provides producer immunity to PSMs [103]. Of note, in the absence of Pmt, PSMs accumulate in the producing cell's cytoplasm with fatal consequences for the cell. The presumable mechanism of Pmt consists in taking up the membrane-active PSMs from within the cytoplasmic membrane and expelling them under ATP hydrolysis, using a mechanism common to energy-driven exporters that provide resistance to membrane-active substances. We are currently performing studies aimed at analyzing whether Pmt has broader substrate specificity, possibly also expelling AMPs. This appears likely, given the amino acid sequence diversity of Pmt's PSM substrates, which virtually only have their secondary structure of amphipathic alpha-helicity in common.

4. Conclusions and outlook

While staphylococci have a variety of resistance mechanisms to AMPs, which also include elaborate sensing and regulatory mechanisms, it is striking that many of those mechanisms have additional biological functions. For example, WTA and PIA/PNAG play very important roles in AMP resistance, but are also necessary for colonization and biofilm formation. Furthermore, the Aps/VraFG sensor/regulator controls many genes not related to AMP resistance. Moreover, many AMP exporters seem to have secondary, or possibly original, functions in providing producer immunity to specific bacteriocins. Finally, staphylococci benefit from the AMP-degrading activity of multiple non-specific secreted proteases, but these proteases certainly have a multitude of other biological functions as well, such as in degrading host tissue for nutrient acquisition. It is thus tempting to speculate that many staphylococcal AMP resistance mechanisms have evolved from other original mechanisms, despite the fact that staphylococci as commensals of humans and other mammals must have been exposed to AMPs for a very long time in evolution. In support of this notion, the factors of protection that many staphylococcal AMP resistance mechanisms provide are rather small. Thus, AMP resistance mechanisms probably need to be revisited with a bigger picture of bacterial pathogenesis and general physiology in mind.

As seen with the lipopeptide daptomycin, which is subject to similar bacterial resistance mechanisms as AMPs, research on staphylococcal AMP resistance mechanisms has significance that goes beyond the mere study of AMP resistance. A more detailed understanding of the mechanisms providing resistance to AMPs and in general, anti-staphylococcal agents, will enhance our preparedness for the development of resistance to clinically important antibiotics. Furthermore, AMPs have often been proposed as novel antibacterial agents, in particular for infections involving biofilms, which are highly resistant to merely bacteriostatic agents. The study of AMP resistance thus also allows us to foresee the spread of potential resistance mechanisms to such agents, should they make it to therapeutic use.

Finally, AMP resistance mechanisms by themselves may represent valuable targets for antimicrobial drug development. One such example is the Pmt exporter, which combines importance as an exporter of key staphylococcal virulence factors with a role in producer immunity and, possibly, AMP resistance [104]. Other possible targets include the widespread bacterial AMP resistance mechanisms of TA D-alanylation (by Dlt) and PG amino-acylation (by MprF and homologues) as well as their control (by Aps).

Transparency documents

The Transparency documents associated with this article can be found, in the online version.

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References

[1] A.C. Uhlemann, M. Otto, F.D. Lowy, F.R. DeLeo, Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*, Infect. Genet. Evol. 21 (2014) 563–574.
[2] F.R. DeLeo, H.F. Chambers, Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era, J. Clin. Invest. 119 (2009) 2464–2474.
[3] R.E. Williams, Healthy carriage of *Staphylococcus aureus*: its prevalence and importance, Bacteriol. Rev. 27 (1963) 56–71.

[4] W.E. Kloos, M.S. Musselwhite, Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin, Appl. Microbiol. 30 (1975) 381–385.
[5] N.H. Eriksen, F. Espersen, V.T. Rosdahl, K. Jensen, Carriage of *Staphylococcus aureus* among 104 healthy persons during a 19-month period, Epidemiol. Infect. 115 (1995) 51–60.
[6] J.A. Kluytmans, H.F. Wertheim, Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections, Infection 33 (2005) 3–8.
[7] C. von Eiff, K. Becker, K. Machka, H. Stammer, G. Peters, Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group, N. Engl. J. Med. 344 (2001) 11–16.
[8] F.D. Lowy, *Staphylococcus aureus* infections, N. Engl. J. Med. 339 (1998) 520–532.
[9] B. Schittek, R. Hipfel, B. Sauer, J. Bauer, H. Kalbacher, S. Stevanovic, M. Schirle, K. Schroeder, N. Blin, F. Meier, G. Rassner, C. Garbe, Dermcidin: a novel human antibiotic peptide secreted by sweat glands, Nat. Immunol. 2 (2001) 1133–1137.
[10] A. Peschel, H.G. Sahl, The co-evolution of host cationic antimicrobial peptides and microbial resistance, Nat. Rev. Microbiol. 4 (2006) 529–536.
[11] M. Li, Y. Lai, A.E. Villaruz, D.J. Cha, D.E. Sturdevant, M. Otto, Gram-positive three-component antimicrobial peptide-sensing system, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 9469–9474.
[12] M. Meehl, S. Herbert, F. Gotz, A. Cheung, Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 51 (2007) 2679–2689.
[13] H.M. Neoh, L. Cui, H. Yuzawa, F. Takeuchi, M. Matsuo, K. Hiramatsu, Mutated response regulator *graR* is responsible for phenotypic conversion of *Staphylococcus aureus* from heterogeneous vancomycin–intermediate resistance to vancomycin–intermediate resistance, Antimicrob. Agents Chemother. 52 (2008) 45–53.
[14] A.L. Cheung, A.S. Bayer, M.R. Yeaman, Y.Q. Xiong, A.J. Waring, G. Memmi, N. Donegan, S. Chaili, S.J. Yang, Site-specific mutation of the sensor kinase, GraS, in *Staphylococcus aureus* alters the adaptive response to distinct cationic antimicrobial peptides, Infect. Immun. 82 (2014) 5336–5345.
[15] M. Li, D.J. Cha, Y. Lai, A.E. Villaruz, D.E. Sturdevant, M. Otto, The antimicrobial peptide-sensing system aps of *Staphylococcus aureus*, Mol. Microbiol. 66 (2007) 1136–1147.
[16] S.J. Yang, A.S. Bayer, N.N. Mishra, M. Meehl, N. Ledala, M.R. Yeaman, Y.Q. Xiong, A.L. Cheung, The *Staphylococcus aureus* two-component regulatory system, GraRS, senses and confers resistance to selected cationic antimicrobial peptides, Infect. Immun. 80 (2012) 74–81.
[17] M. Falord, U. Mader, A. Hiron, M. Debarbouille, T. Msadek, Investigation of the *Staphylococcus aureus* GraSR regulon reveals novel links to virulence, stress response and cell wall signal transduction pathways, PLoS ONE 6 (2011) e21323.
[18] M. Falord, G. Karimova, A. Hiron, T. Msadek, GraXSR proteins interact with the VraFG ABC transporter to form a five-component system required for cationic antimicrobial peptide sensing and resistance in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 56 (2012) 1047–1058.
[19] G.Y. Cheung, K. Rigby, R. Wang, S.Y. Queck, K.R. Braughton, A.R. Whitney, M. Teintze, F.R. DeLeo, M. Otto, *Staphylococcus epidermidis* strategies to avoid killing by human neutrophils, PLoS Pathog. 6 (2010) e1001133.
[20] Y. Lai, A.E. Villaruz, M. Li, D.J. Cha, D.E. Sturdevant, M. Otto, The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in staphylococci, Mol. Microbiol. 63 (2007) 497–506.
[21] M. Fridman, G.D. Williams, U. Muzamal, H. Hunter, K.W. Siu, D. Golemi-Kotra, Two unique phosphorylation-driven signaling pathways crosstalk in *Staphylococcus aureus* to modulate the cell-wall charge: Stk1/Stp1 meets GraSR, Biochemistry 52 (2013) 7975–7986.
[22] S.J. Yang, Y.Q. Xiong, M.R. Yeaman, K.W. Bayles, W. Abdelhady, A.S. Bayer, Role of the LytSR two-component regulatory system in adaptation to cationic antimicrobial peptides in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 57 (2013) 3875–3882.
[23] A. Peschel, R.W. Jack, M. Otto, L.V. Collins, P. Staubitz, G. Nicholson, H. Kalbacher, W.F. Nieuwenhuizen, G. Jung, A. Tarkowski, K.P. van Kessel, J.A. van Strijp, *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine, J. Exp. Med. 193 (2001) 1067–1076.
[24] S. Klein, C. Lorenzo, S. Hoffmann, J.M. Walther, S. Storbeck, T. Piekarski, B.J. Tindall, V. Wray, M. Nimtz, J. Moser, Adaptation of *Pseudomonas aeruginosa* to various conditions includes tRNA-dependent formation of alanyl-phosphatidylglycerol, Mol. Microbiol. 71 (2009) 551–565.
[25] N.C. Johnston, J.K. Baker, H. Goldfine, Phospholipids of *Clostridium perfringens*: a reexamination, FEMS Microbiol. Lett. 233 (2004) 65–68.
[26] C.M. Ernst, P. Staubitz, N.N. Mishra, S.J. Yang, G. Hornig, H. Kalbacher, A.S. Bayer, D. Kraus, A. Peschel, The bacterial defensin resistance protein MprF consists of separable domains for lipid lysinylation and antimicrobial peptide repulsion, PLoS Pathog. 5 (2009) e1000660.
[27] C.J. Slavetinsky, A. Peschel, C.M. Ernst, Alanyl-phosphatidylglycerol and lysyl-phosphatidylglycerol are translocated by the same MprF flippases and have similar capacities to protect against the antibiotic daptomycin in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 56 (2012) 3492–3497.
[28] L. Friedman, J.D. Alder, J.A. Silverman, Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 50 (2006) 2137–2145.
[29] K. Julian, K. Kosowska-Shick, C. Whitener, M. Roos, H. Labischinski, A. Rubio, L. Parent, L. Ednie, L. Koeth, T. Bogdanovich, P.C. Appelbaum, Characterization of a daptomycin-nonsusceptible vancomycin-intermediate *Staphylococcus aureus* strain in a patient with endocarditis, Antimicrob. Agents Chemother. 51 (2007) 3445–3448.
[30] M.H. Murthy, M.E. Olson, R.W. Wickert, P.D. Fey, Z. Jalali, Daptomycin non-susceptible meticillin-resistant *Staphylococcus aureus* USA 300 isolate, J. Med. Microbiol. 57 (2008) 1036–1038.
[31] K. Kosowska-Shick, C. Clark, G.A. Pankuch, P. McGhee, B. Dewasse, L. Beachel, P.C. Appelbaum, Activity of telavancin against staphylococci and enterococci determined by MIC and resistance selection studies, Antimicrob. Agents Chemother. 53 (2009) 4217–4224.
[32] S.J. Yang, Y.Q. Xiong, P.M. Dunman, J. Schrenzel, P. Francois, A. Peschel, A.S. Bayer, Regulation of *mprF* in daptomycin-nonsusceptible *Staphylococcus aureus* strains, Antimicrob. Agents Chemother. 53 (2009) 2636–2637.
[33] A.S. Bayer, N.N. Mishra, G. Sakoulas, P. Nonejuie, C.C. Nast, J. Pogliano, K.T. Chen, S.N. Ellison, M.R. Yeaman, S.J. Yang, Heterogeneity of *mprF* sequences in methicillin-resistant *Staphylococcus aureus* clinical isolates: role in cross-resistance between daptomycin and host defense antimicrobial peptides, Antimicrob. Agents Chemother. 58 (2014) 7462–7467.
[34] A.M. Smith, J.S. Harrison, K.M. Sprague, H. Roy, A conserved hydrolase responsible for the cleavage of aminoacylphosphatidylglycerol in the membrane of *Enterococcus faecium*, J. Biol. Chem. 288 (2013) 22768–22776.
[35] W. Arendt, M.K. Groenewold, S. Hebecker, J.S. Dickschat, J. Moser, Identification and characterization of a periplasmic aminoacyl-phosphatidylglycerol hydrolase responsible for *Pseudomonas aeruginosa* lipid homeostasis, J. Biol. Chem. 288 (2013) 24717–24730.
[36] J.J. Armstrong, J. Baddiley, J.G. Buchanan, B. Carss, G.R. Greenberg, Isolation and structure of ribitol phosphate derivatives (teichoic acids) from bacterial cell walls, J. Chem. Soc. (1958) 4344–4354.
[37] A. Bera, R. Biswas, S. Herbert, E. Kulauzovic, C. Weidenmaier, A. Peschel, F. Gotz, Influence of wall teichoic acid on lysozyme resistance in *Staphylococcus aureus*, J. Bacteriol. 189 (2007) 280–283.
[38] N. Kojima, Y. Araki, E. Ito, Structure of the linkage units between ribitol teichoic acids and peptidoglycan, J. Bacteriol. 161 (1985) 299–306.
[39] G. Xia, T. Kohler, A. Peschel, The wall teichoic acid and lipoteichoic acid polymers of *Staphylococcus aureus*, Int. J. Med. Microbiol. 300 (2010) 148–154.
[40] S. Brown, J.P. Santa Maria Jr., S. Walker, Wall teichoic acids of gram-positive bacteria, Annu. Rev. Microbiol. 67 (2013) 313–336.
[41] M.G. Percy, A. Grundling, Lipoteichoic acid synthesis and function in gram-positive bacteria, Annu. Rev. Microbiol. 68 (2014) 81–100.
[42] O. Schneewind, D. Missiakas, Lipoteichoic acids, phosphate-containing polymers in the envelope of gram-positive bacteria, J. Bacteriol. 196 (2014) 1133–1142.
[43] C. Weidenmaier, A. Peschel, Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions, Nat. Rev. Microbiol. 6 (2008) 276–287.
[44] A. Peschel, M. Otto, R.W. Jack, H. Kalbacher, G. Jung, F. Gotz, Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides, J. Biol. Chem. 274 (1999) 8405–8410.
[45] M.P. Heaton, F.C. Neuhaus, Biosynthesis of D-alanyl-lipoteichoic acid: cloning, nucleotide sequence, and expression of the *Lactobacillus casei* gene for the D-alanine-activating enzyme, J. Bacteriol. 174 (1992) 4707–4717.
[46] M.P. Heaton, F.C. Neuhaus, Role of the D-alanyl carrier protein in the biosynthesis of D-alanyl-lipoteichoic acid, J. Bacteriol. 176 (1994) 681–690.
[47] M. Perego, P. Glaser, A. Minutello, M.A. Strauch, K. Leopold, W. Fischer, Incorporation of D-alanine into lipoteichoic acid and wall teichoic acid in *Bacillus subtilis*, identification of genes and regulation, J. Biol. Chem. 270 (1995) 15598–15606.
[48] F.C. Neuhaus, J. Baddiley, A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria, Microbiol. Mol. Biol. Rev. 67 (2003) 686–723.
[49] H.U. Koch, R. Doker, W. Fischer, Maintenance of D-alanine ester substitution of lipoteichoic acid by reesterification in *Staphylococcus aureus*, J. Bacteriol. 164 (1985) 1211–1217.
[50] T. Koprivnjak, V. Mlakar, L. Swanson, B. Fournier, A. Peschel, J.P. Weiss, Cation-induced transcriptional regulation of the *dlt* operon of *Staphylococcus aureus*, J. Bacteriol. 188 (2006) 3622–3630.
[51] M.L. Atilano, P.M. Pereira, J. Yates, P. Reed, H. Veiga, M.G. Pinho, S.R. Filipe, Teichoic acids are temporal and spatial regulators of peptidoglycan cross-linking in *Staphylococcus aureus*, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 18991–18996.
[52] M. Schlag, R. Biswas, B. Krismer, T. Kohler, S. Zoll, W. Yu, H. Schwarz, A. Peschel, F. Gotz, Role of staphylococcal wall teichoic acid in targeting the major autolysin Atl, Mol. Microbiol. 75 (2010) 864–873.
[53] A. Qamar, D. Golemi-Kotra, Dual roles of FmtA in *Staphylococcus aureus* cell wall biosynthesis and autolysis, Antimicrob. Agents Chemother. 56 (2012) 3797–3805.
[54] T. Koprivnjak, C. Weidenmaier, A. Peschel, J.P. Weiss, Wall teichoic acid deficiency in *Staphylococcus aureus* confers selective resistance to mammalian group IIA phospholipase A(2) and human beta-defensin 3, Infect. Immun. 76 (2008) 2169–2176.
[55] V. Winstel, C. Liang, P. Sanchez-Carballo, M. Steglich, M. Munar, B.M. Broker, J.R. Penades, U. Nubel, O. Holst, T. Dandekar, A. Peschel, G. Xia, Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens, Nat. Commun. 4 (2013) 2345.
[56] C. Weidenmaier, J.F. Kokai-Kun, S.A. Kristian, T. Chanturiya, H. Kalbacher, M. Gross, G. Nicholson, B. Neumeister, J.J. Mond, A. Peschel, Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections, Nat. Med. 10 (2004) 243–245.
[57] S. Baur, M. Rautenberg, M. Faulstich, T. Grau, Y. Severin, C. Unger, W.H. Hoffmann, T. Rudel, I.B. Autenrieth, C. Weidenmaier, A nasal epithelial receptor for *Staphylococcus aureus* WTA governs adhesion to epithelial cells and modulates nasal colonization, PLoS Pathog. 10 (2014) e1004089.

[58] D. Mack, W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, R. Laufs, The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear beta-1,6-linked glucosaminoglycan: purification and structural analysis, J. Bacteriol. 178 (1996) 175–183.
[59] S.E. Cramton, C. Gerke, N.F. Schnell, W.W. Nichols, F. Gotz, The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation, Infect. Immun. 67 (1999) 5427–5433.
[60] T. Maira-Litran, A. Kropec, C. Abeygunawardana, J. Joyce, G. Mark 3rd, D.A. Goldmann, G.B. Pier, Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide, Infect. Immun. 70 (2002) 4433–4440.
[61] C. Heilmann, O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, F. Gotz, Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*, Mol. Microbiol. 20 (1996) 1083–1091.
[62] X. Wang, J.F.I. Preston, T. Romeo, The *pgaABCD* locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation, J. Bacteriol. 186 (2004) 2724–2734.
[63] M. Otto, Staphylococcal biofilms, Curr. Top. Microbiol. Immunol. 322 (2008) 207–228.
[64] C. Vuong, S. Kocianova, J.M. Voyich, Y. Yao, E.R. Fischer, F.R. DeLeo, M. Otto, A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence, J. Biol. Chem. 279 (2004) 54881–54886.
[65] J.L. Brooks, K.K. Jefferson, Phase variation of poly-N-acetylglucosamine expression in *Staphylococcus aureus*, PLoS Pathog. 10 (2014) e1004292.
[66] F.B. Oppermann-Sanio, A. Steinbuchel, Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production, Naturwissenschaften 89 (2002) 11–22.
[67] S. Kocianova, C. Vuong, Y. Yao, J.M. Voyich, E.R. Fischer, F.R. DeLeo, M. Otto, Key role of poly-gamma-DL-glutamic acid in immune evasion and virulence of *Staphylococcus epidermidis*, J. Clin. Invest. 115 (2005) 688–694.
[68] C. Vuong, J.M. Voyich, E.R. Fischer, K.R. Braughton, A.R. Whitney, F.R. DeLeo, M. Otto, Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system, Cell. Microbiol. 6 (2004) 269–275.
[69] A.I. Spiliopoulou, M.I. Krevvata, F. Kolonitsiou, L.G. Harris, T.S. Wilkinson, A.P. Davies, G.O. Dimitracopoulos, N.K. Karamanos, D. Mack, E.D. Anastassiou, An extracellular *Staphylococcus epidermidis* polysaccharide: relation to polysaccharide intercellular adhesin and its implication in phagocytosis, BMC Microbiol. 12 (2012) 76.
[70] Y. Yao, D.E. Sturdevant, M. Otto, Genomewide analysis of gene expression in *Staphylococcus epidermidis* biofilms: insights into the pathophysiology of *S. epidermidis* biofilms and the role of phenol-soluble modulins in formation of biofilms, J. Infect. Dis. 191 (2005) 289–298.
[71] M.E. Rupp, J.S. Ulphani, P.D. Fey, K. Bartscht, D. Mack, Characterization of the importance of polysaccharide intercellular adhesin/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model, Infect. Immun. 67 (1999) 2627–2632.
[72] G.R. Drapeau, Y. Boily, J. Houmard, Purification and properties of an extracellular protease of *Staphylococcus aureus*, J. Biol. Chem. 247 (1972) 6720–6726.
[73] M. Sieprawska-Lupa, P. Mydel, K. Krawczyk, K. Wojcik, M. Puklo, B. Lupa, P. Suder, J. Silberring, M. Reed, J. Pohl, W. Shafer, F. McAleese, T. Foster, J. Travis, J. Potempa, Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases, Antimicrob. Agents Chemother. 48 (2004) 4673–4679.
[74] G.R. Drapeau, Role of metalloprotease in activation of the precursor of staphylococcal protease, J. Bacteriol. 136 (1978) 607–613.
[75] P. Teufel, F. Gotz, Characterization of an extracellular metalloprotease with elastase activity from *Staphylococcus epidermidis*, J. Bacteriol. 175 (1993) 4218–4224.
[76] G. Dubin, D. Chmiel, P. Mak, M. Rakwalska, M. Rzychon, A. Dubin, Molecular cloning and biochemical characterisation of proteases from *Staphylococcus epidermidis*, Biol. Chem. 382 (2001) 1575–1582.
[77] J.L. Moon, A. Banbula, A. Oleksy, J.A. Mayo, J. Travis, Isolation and characterization of a highly specific serine endopeptidase from an oral strain of *Staphylococcus epidermidis*, Biol. Chem. 382 (2001) 1095–1099.
[78] P.F. Chan, S.J. Foster, Role of SarA in virulence determinant production and environmental signal transduction in *Staphylococcus aureus*, J. Bacteriol. 180 (1998) 6232–6241.
[79] P.M. Dunman, E. Murphy, S. Haney, D. Palacios, G. Tucker-Kellogg, S. Wu, E.L. Brown, R.J. Zagursky, D. Shlaes, S.J. Projan, Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the *agr* and/or *sarA* loci, J. Bacteriol. 183 (2001) 7341–7353.
[80] K. Rice, R. Peralta, D. Bast, J. de Azavedo, M.J. McGavin, Description of staphylococcus serine protease (ssp.) operon in *Staphylococcus aureus* and nonpolar inactivation of *sspA*-encoded serine protease, Infect. Immun. 69 (2001) 159–169.
[81] T. Jin, M. Bokarewa, T. Foster, J. Mitchell, J. Higgins, A. Tarkowski, *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism, J. Immunol. 172 (2004) 1169–1176.
[82] M. Bokarewa, A. Tarkowski, Human alpha-defensins neutralize fibrinolytic activity exerted by staphylokinase, Thromb. Haemost. 91 (2004) 991–999.
[83] D.B. Baruah, R.N. Dash, M.R. Chaudhari, S.S. Kadam, Plasminogen activators: a comparison, Vasc. Pharmacol. 44 (2006) 1–9.
[84] M.H. Braff, A.L. Jones, S.J. Skerrett, C.E. Rubens, *Staphylococcus aureus* exploits cathelicidin antimicrobial peptides produced during early pneumonia to promote staphylokinase-dependent fibrinolysis, J. Infect. Dis. 195 (2007) 1365–1372.
[85] A. Hollands, D. Gonzalez, E. Leire, C. Donald, R.L. Gallo, M. Sanderson-Smith, P.C. Dorrestein, V. Nizet, A bacterial pathogen co-opts host plasmin to resist killing by cathelicidin antimicrobial peptides, J. Biol. Chem. 287 (2012) 40891–40897.
[86] V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D.S. Weiss, Y. Weinrauch, A. Zychlinsky, Neutrophil extracellular traps kill bacteria, Science 303 (2004) 1532–1535.
[87] V. Thammavongsa, D.M. Missiakas, O. Schneewind, *Staphylococcus aureus* degrades neutrophil extracellular traps to promote immune cell death, Science 342 (2013) 863–866.
[88] R. Doshi, D.A. Gutmann, Y.S. Khoo, L.A. Fagg, H.W. van Veen, The choreography of multidrug export, Biochem. Soc. Trans. 39 (2011) 807–811.
[89] M. Otto, F. Gotz, ABC transporters of staphylococci, Res. Microbiol. 152 (2001) 351–356.
[90] S. Rieg, A. Huth, H. Kalbacher, W.V. Kern, Resistance against antimicrobial peptides is independent of *Escherichia coli* AcrAB, *Pseudomonas aeruginosa* MexAB and *Staphylococcus aureus* NorA efflux pumps, Int. J. Antimicrob. Agents 33 (2009) 174–176.
[91] G. Bierbaum, H.G. Sahl, Lantibiotics: mode of action, biosynthesis and bioengineering, Curr. Pharm. Biotechnol. 10 (2009) 2–18.
[92] F. Gotz, S. Perconti, P. Popella, R. Werner, M. Schlag, Epidermin and gallidermin: staphylococcal lantibiotics, Int. J. Med. Microbiol. 304 (2014) 63–71.
[93] S. Gebhard, ABC transporters of antimicrobial peptides in *Firmicutes* bacteria — phylogeny, function and regulation, Mol. Microbiol. 86 (2012) 1295–1317.
[94] M. Otto, A. Peschel, F. Gotz, Producer self-protection against the lantibiotic epidermin by the ABC transporter EpiFEG of *Staphylococcus epidermidis* Tu3298, FEMS Microbiol. Lett. 166 (1998) 203–211.
[95] A. Peschel, N. Schnell, M. Hille, K.D. Entian, F. Gotz, Secretion of the lantibiotics epidermin and gallidermin: sequence analysis of the genes *gdmT* and *gdmH*, their influence on epidermin production and their regulation by EpiQ, Mol. Gen. Genet. 254 (1997) 312–318.
[96] A. Hiron, M. Falord, J. Valle, M. Debarbouille, T. Msadek, Bacitracin and nisin resistance in *Staphylococcus aureus*: a novel pathway involving the BraS/BraR two-component system (SA2417/SA2418) and both the BraD/BraE and VraD/VraE ABC transporters, Mol. Microbiol. 81 (2011) 602–622.
[97] Y. Yoshida, M. Matsuo, Y. Oogai, F. Kato, N. Nakamura, M. Sugai, H. Komatsuzawa, Bacitracin sensing and resistance in *Staphylococcus aureus*, FEMS Microbiol. Lett. 320 (2011) 33–39.
[98] S.L. Kolar, V. Nagarajan, A. Oszmiana, F.E. Rivera, H.K. Miller, J.E. Davenport, J.T. Riordan, J. Potempa, D.S. Barber, J. Koziel, M.O. Elasri, L.N. Shaw, NsaRS is a cell-envelope-stress-sensing two-component system of *Staphylococcus aureus*, Microbiology 157 (2011) 2206–2219.
[99] T. Mascher, Intramembrane-sensing histidine kinases: a new family of cell envelope stress sensors in Firmicutes bacteria, FEMS Microbiol. Lett. 264 (2006) 133–144.
[100] R. Bernard, A. Guiseppi, M. Chippaux, M. Foglino, F. Denizot, Resistance to bacitracin in *Bacillus subtilis*: unexpected requirement of the BceAB ABC transporter in the control of expression of its own structural genes, J. Bacteriol. 189 (2007) 8636–8642.
[101] A. Peschel, M. Otto, Phenol-soluble modulins and staphylococcal infection, Nat. Rev. Microbiol. 11 (2013) 667–673.
[102] H.S. Joo, G.Y. Cheung, M. Otto, Antimicrobial activity of community-associated methicillin-resistant *Staphylococcus aureus* is caused by phenol-soluble modulin derivatives, J. Biol. Chem. 286 (2011) 8933–8940.
[103] S.S. Chatterjee, H.S. Joo, A.C. Duong, T.D. Dieringer, V.Y. Tan, Y. Song, E.R. Fischer, G.Y. Cheung, M. Li, M. Otto, Essential *Staphylococcus aureus* toxin export system, Nat. Med. 19 (2013) 364–367.
[104] S.S. Chatterjee, M. Otto, How can *Staphylococcus aureus* phenol-soluble modulins be targeted to inhibit infection? Future Microbiol 8 (2013) 693–696.