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# CRUCIAL ROLE OF MPRF-MEDIATED DEFENSIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* NASAL COLONIZATION

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## SUMMARY

Major bacterial pathogens such as *Staphylococcus aureus* are facultative pathogens and are members of the microbiotas of human body surfaces. *S. aureus* uses the MprF protein to achieve resistance to host-derived cationic antimicrobial peptides (CAMPs) such as defensins and cathelicidins. While the importance of MprF for *S. aureus* invasive infections is well documented it remains unclear if CAMP resistance is also a prerequisite for the capacity of *S. aureus* to colonize its preferred niche in healthy humans, the anterior nares. The cotton rat model of nasal colonization was used to investigate the potential role of MprF as a colonization factor. Of note, a *S. aureus mprF* mutant turned out to have a reduced capacity to colonize cotton rat noses compared to the parental strain. This finding underscores the role of CAMPs in shaping the composition of mirobiotas at mammalian body surfaces and they demonstrate that CAMP resistance mechanisms are promising targets structures for antimicrobials that would not only interfere with infection but also with colonization by bacterial pathogens.

## INTRODUCTION

Bacterial pathogens with resistance to the most effective available antibiotics are increasingly spreading around the world causing severe morbidity and a vast number of deaths. Methicillin-resistant *Staphylococcus aureus* (MRSA) remains the most frequent cause of antibiotic-resistant infections on intensive care units followed by vancomycin-resistant enterococci and enterobacteria producing extended-spectrum b-lactamases (ESBL) or carbapenemases, which have emerged in recent years and are on the rise (*Boucher* et al., 2009). Of note, most of these pathogens have their reservoirs in the microbiotas of human body surfaces such as the intestine (enterobacteria) or nose (*S. aureus*). Only certain individuals are colonized by such antibiotic-resistant pathogens and colonized patients at risk e.g. on intensive-care units or under immunosuppression often suffer from severe infections caused by an endogenous strain (*Weidenmaier* et al., 2012; *Biehl* et al., 2014). Why only certain persons are colonized and how antibiotic-resistant bacteria can prevail in human microbiotas has remained unclear.

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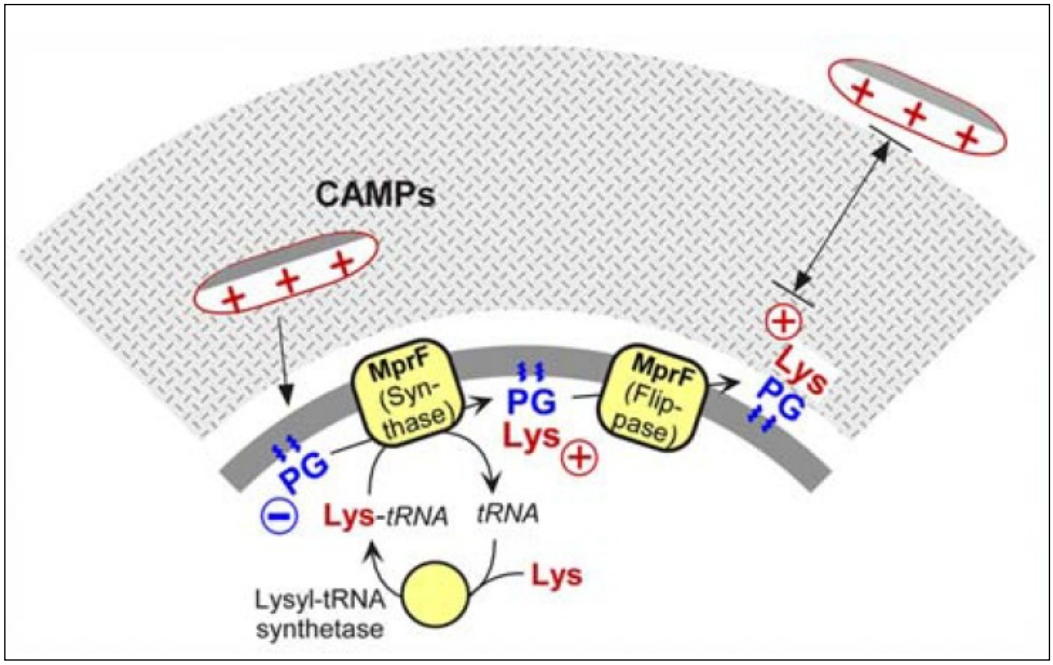


**Figure 1**: CAMP resistance mediated by MprF. (From: Ernst and Peschel A, 2011).

The capacity of a given bacterial strain to persist in a microbial community is governed by its competitive fitness in the interaction with other microbes and with the host. The underlying mechanisms are complex and have been hardly explored. They may include capabilities to (i) bind to a limited number of host attachment sites, (ii) take up and metabolise scant nutrients, or (iii) produce antibacterial substances referred to as bacteriocins that inhibit competitors. In addition, (iv) intrinsic mechanisms conferring resistance to host antimicrobial peptides such as defensins may contribute to bacterial fitness in the human nose.

Defensin-like 'endogenous antibiotics' include defensins, cathelicidins, RNase 7, or dermcidin (*Hancock* and *Sahl*, 2006). They play key roles in human antibacterial host defence and have also been found to shape the microbiota in the human gut (*Salzman* et al., 2010). Such peptides are released by immigrating neutrophils and secreted e.g. by epithelial cells. Most human antimicrobial peptides have a cationic net charge, which increases their affinity for the generally anionic bacterial cell surface molecules (*Peschel* and *Sahl*, 2006). Such cationic antimicrobial peptides (CAMPs) kill bacteria by damaging essential membrane-associated machineries (*Wenzel* et al., 2014). Peptides with related physicochemical properties and modes of action are also produced by bacteria of human microbiotas (referred to as bacteriocins; e.g. epidermin) (*Arnison* et al., 2013) or are used as therapeutic antibiotics (e.g. daptomycin) (*Bayer* et al., 2013). Antimicrobial peptides produced by human or bacterial cells have been described also in the fluids found in the human nose (*Cole* et al., 1999) but it remains unclear how important they may be for the ecology of the nasal microbial habitat.

Pathogens often have mechanisms to resist antimicrobial peptides and such mechanisms are crucial for resist-

ing bacterial capacities to withstand host defence (*Peschel* and *Sahl*, 2006). *S. aureus* uses several intrinsic mechanisms for resisting CAMPs. They are based on modification of polyanionic cell envelope components such as teichoic acids, membrane lipids, or peptidoglycan to reduce the affinity for host antimicrobials by altering the surface net charge (*Peschel* et al., 1999, 2001; *Munch* et al., 2012). The underlying mechanisms have been found to be crucial for the capacity of *S. aureus* and many other pathogens to colonize or cause infections. The multiple peptide resistance factor MprF is a bifunctional membrane protein that (i) modifies anionic phosphatidylglycerol (PG) with lysine to produce lysyl-PG and (ii) translocates Lys-PG from the inner to the outer leaflet of the cytoplasmic membrane (Figure 1) (*Ernst* et al., 2009). MprF is found in many Gram-positive (staphylococci, listeria, group-B streptococci) and Gram-negative pathogens (pseudomonads, burkholderiae, many plant pathogens such as *Agrobacterium*) and is thought to be an attractive target for future anti-virulence therapeutics (*Ernst* and *Peschel*, 2011). MprF mutants of *S. aureus* and other pathogens are profoundly virulence-attenuated but a potential role of this protein in colonization of human body sites has remained unknown.

## MATERIALS AND METHODS

The cotton rat model was used as described earlier (*Baur* et al, 2014; *Krismer* et al., 2014). Briefly, cotton rats (*Sigmodon hispidus*) were anesthetized and instilled intranasally with 10 µl of $1\times10^8$ colony-forming units (CFU) of *S. aureus*. Six days after bacterial instillation the animals were euthanized and noses were removed surgically. The noses were vortexed in 1 ml of PBS containing 0.5% tween for 30 s. Samples were plated on appropriate agar plates [B-medium, sheep blood containing 250 µg/ml streptomycin and HiCrome Aureus Agar (Fluka)] and the bacterial CFU was determined. All animals received drinking water with 2.5 mg/ml streptomycin continuously, starting three days prior to the experiment to reduce the natural nasal flora. All guidelines of the US Department of Agriculture and the Biosynexus Inc. Institutional Animal Care and Use Committee were followed.

## RESULTS AND DISCUSSION

When inoculated with *S. aureus* the noses of mice quickly loose the bacteria and stable colonization can hardly be established in these animals. In contrast, cotton rats are susceptible to many human respiratory pathogens and the histology of the cotton rat nares is similar to that in humans, as they both have squamous and pseudostratified epithelial areas (*Burian* et al., 2010). Several *S. aureus* factors have been described as determinants for nasal colonization using this model: wall teichoic acid (WTA), a surface-exposed structure of Gram-positive bacteria (*Weidenmaier* et al., 2004), the iron-regulated surface determinant A (encoded by isdA), catalase, alkyl hydroperoxide reductase, the autolysin SceD (encoded by sceD) (*Clarke* et al., 2006; *Cosgrove* et al., 2007), and the methionine-biosynthetic enzyme MetI (*Krismer* et al.,

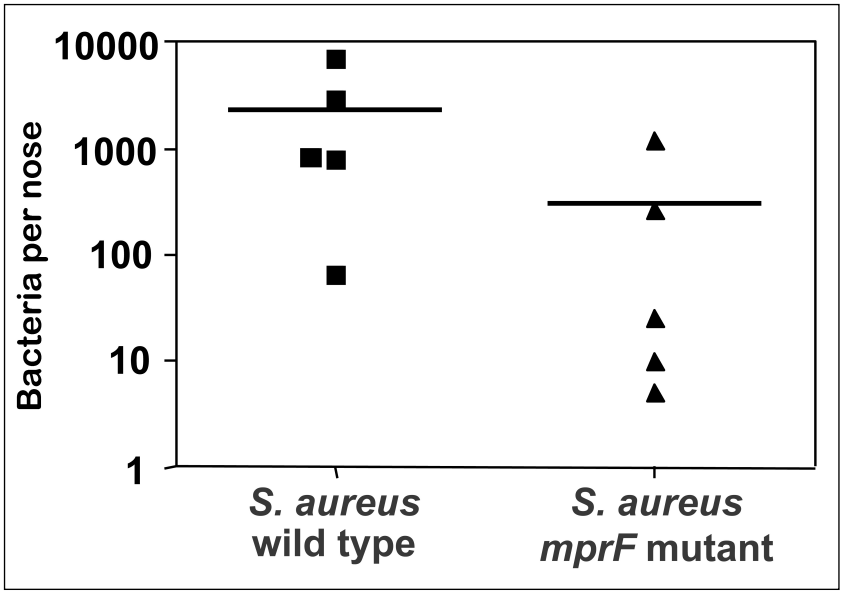


**Figure 2**: Impact of MprF on *S. aureus* nasal colonization in cotton rats.

2014). Several key virulence and colonization factors were found to be expressed at similar levels in human and cotton rat noses (*Burian* et al., 2010; *Krismer* et al., 2014) and antibodies directed against the epithelial cell receptor SREC I block *S. aureus* binding to human and cotton rat nasal epithelial cells (*Baur* et al., 2014). All these evidences indicate that the cotton rat represents a reliable and representative model for experimental nasal colonization studies.

In order to study if MprF may play a role in *S. aureus* nasal colonization the recently described *S. aureus* SA113 *mprF* deletion mutant (*Peschel* et al., 2001) and its parental wild-type strain were instilled into the noses of cotton rats and the numbers of *S. aureus* bacteria per nose were determined seven days later (Figure 2). While the wild type colonized the animals at numbers of several thousand bacteria per nose the *mprF* mutant was recovered at ca. ten times lower numbers. Thus, the MprF-mediated resistance mechanism enables *S. aureus* to persist in the nose while its absence leads to a strongly abrogated colonization capacity.

Defensins and other CAMPs are found in nasal fluids of *S. aureus*-colonized humans but it has remained unknown if they contribute to limiting the growth of nasal bacteria. Our results indicate that MprF is not only avirulence but also an important colonization factor for *S. aureus*. The occurrence of MprF in many other human commensals, opportunists, and aggressive pathogens suggests that it may be a general bacterial mechanism allowing growth in host environments with high CAMP concentrations. It remains unclear if only host-derived CAMPs limit bacterial growth in the nose or if bacteriocins produced by other bacteria of the nasal microbiota may also be a reason for *S. aureus* to require CAMP resistance mechanism during its commensal life style. A second CAMP resistance mechanism, the teichoic acid-modifying DltABCD machinery, has also been found to contribute to the nasal colonization capacity of *S. aureus* (*Weidenmaier* et al., 2004).

However, since teichoic acids also play a critical role in the attachment of *S. aureus* to nasal epithelia cells the potential importance of DltABCD-dependent CAMP resistance for *S. aureus* nasal colonization has remained unknown.

*S. aureus* carriage in ca. 30% of the human population represents a major risk factor for severe invasive infections (*von Eiff* et al., 2001). Therefore, patients are decolonized with the antibiotic mupirocin before they are admitted to elective surgery. With increasing resistance to mupirocin among clinical *S. aureus* alternative antibiotics are urgently needed (*Arnison* et al., 2013; *Hetem* and Bonten, 2013). Our discovery of a crucial role of MprF in nasal colonization puts MprF on the list of potential targets for new antimicrobials that would not kill *S. aureus* directly but render the bacteria susceptible to endogenous human CAMPs, bacteriocins, and antibiotics such as daptomycin.

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