Antimicrobial Activity of Community-associated Methicillin-resistant *Staphylococcus aureus* Is Caused by Phenol-soluble Modulin Derivatives*

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Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are causing an ongoing pandemic of mostly skin and soft tissue infections. The success of CA-MRSA as pathogens is due to a combination of antibiotic resistance with high virulence. In addition, it has been speculated that CA-MRSA strains such as the epidemic U.S. clone USA300 have increased capacity to colonize human epithelia, owing to bacteriocin-based bacterial interference. We here analyzed the molecular basis of antimicrobial activity detected in *S. aureus* strains, including those of the USA300 lineage. In contrast to a previous hypothesis, we found that this activity is not due to expression of a lantibiotic-type bacteriocin, but proteolytically processed derivatives of the phenol-soluble modulin (PSM) peptides PSMα1 and PSMα2. Notably, processed PSMα1 and PSMα2 exhibited considerable activity against *Streptococcus pyogenes*, indicating a role of PSMs in the interference of *S. aureus* strains with the competing colonizing pathogen. Furthermore, by offering a competitive advantage during colonization of the human body, the characteristically high production of PSMs in USA300 and other CA-MRSA strains may thus contribute not only to virulence but also the exceptional capacity of those strains to sustainably spread in the population, which so far has remained poorly understood.

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maturation and detachment (20), which has been demonstrated for the β-type PSMs of S. epidermidis (21). In addition, it has recently been shown that specific S. epidermidis PSMs, S. epidermidis PSMδ and δ-toxin (PSMγ), have antimicrobial activities against Streptococcus pyogenes owing to their capacity to form pores in the bacterial cell membrane (22). Finally, antimicrobial activities have been detected sporadically for other staphylococcal PSM-like peptides (23). Whether S. aureus PSMs are antimicrobial and responsible for antimicrobial activities exhibited by some S. aureus strains is unknown. Importantly, PSMs are produced in high amounts in the USA300/USA500 lineage of the S. aureus clonal complex 8 (17, 24). This is believed to be due to high activity of the agr quorum-sensing system, which strictly regulates PSM production (17, 25).

Here, we present a comprehensive analysis of antimicrobial activities of all known S. aureus PSM peptides. By construction and use of psm and adm deletion strains and purification of the antimicrobial activity of the USA300 strain, we demonstrate that PSM-derived peptides rather than the Adm2 antibiotic are the source of that activity, suggesting a potential contribution of PSMs to the proposed competitive ecological advantage of CA-MRSA.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions**—Bacterial strains used in this study are shown in Table 1. All cultures were grown in tryptic soy broth (TSB) at 37 °C with shaking at 180 rpm, unless otherwise noted.

**Peptide Synthesis**—Peptides were ordered through the Peptide Synthesis Unit of the NIAID and synthesized by commercial vendors. All PSM peptides used were N-formylated, unless otherwise noted.

**Production of Multiple psm Deletion Mutants**—To produce multiple psm deletion mutants in S. aureus LAC, plasmid pKOR1pmcwa was constructed to delete the psmcwa operon without introduction of an antibiotic resistance cassette. To that end, two PCR products were amplified from chromosomal DNA of strain LAC using primers psmcwa1 and psmcwaε1, and psmcwa2 and psmcwa2ε, respectively (Table 2). The two PCR products were cleaved using EcoRI and ligated, and the ligated product was cloned into plasmid pKOR1 (26) using the Invitrogen Clonase kit. Then, the resulting plasmid pKOR1pmcwa was transformed first into S. aureus RN4220 and finally S. aureus LAC hld and S. aureus LAC psmδ mutants (17). Afterward, allelic replacement and screening procedures were performed as described (26). The resulting S. aureus LAC psmδ/psmδ double mutant was transformed with plasmid pKOR1hld (17), and allelic replacement was performed as described (26), resulting in the psm triple mutant S. aureus LAC psmδ/psmδ/hld, in which all known psm loci are deleted, or, in the case of the δ-toxin gene hld, the start codon is mutated to abolish translation of hld (17) without interfering with the function of the regulatory RNA, RNAIII, in which hld is embedded (27).

**Production of adm Deletion Mutant**—To abolish Adm production in strain USA300, the entire adm cluster (from the admA2 to the admG gene, comprising all genes involved in posttranslational modification, processing, and producer immunity in addition to the admA2 structural gene) was deleted using a procedure based on the temperature-sensitive plasmid pBT2 (28). To that end, two PCR products flanking the adm operon were amplified from genomic DNA of strain USA300 (LAC) using primers admKODownFor and adm-

![Table 1](image-url)

**TABLE 1**

| Strain                       | Characteristics                          | Source/Ref. |
|------------------------------|------------------------------------------|-------------|
| S. aureus                    |                                          |             |
| USA300 (LAC)                 | Epidemic U.S. CA-MRSA clone, strain LAC, ST8, CC8 | Ref. 36     |
| USA300 (LAC) psmcwa          | LAC psmcwa deletion mutant; specA         | Ref. 17     |
| USA300 (LAC) psmδ            | LAC psmδ deletion mutant; specB          | Ref. 17     |
| USA300 (LAC) hld             | LAC hld deletion mutant; hld start codon changed from ATG to ATT | Ref. 17     |
| USA300 (LAC) psmcwa/psmδ     | LAC psmcwa/psmδ deletion mutant; specC    | Ref. 17     |
| USA300 (LAC) psmcwa/hld      | LAC psmcwa/hld deletion mutant; specD     | This study  |
| USA300 (LAC) agr             | LAC agr deletion mutant; tetA             | Ref. 17     |
| USA300 (LAC) adm             | LAC adm deletion mutant; specE            | This study  |
| USA300 (SF8300)              | Epidemic U.S. CA-MRSA clone, strain SF8300 | Ref. 24     |
| USA500 (BD02-25)             | Endemic HA-MRSA clone, progenitor of USA300, ST8, CC8 | Ref. 24     |
| Archaic strain (COL)         | HA-MRSA, ST250, CC8                      | Ref. 24     |
| Iberian strain (BAA44)       | HA-MRSA, ST247, CC8                      | Ref. 24     |
| Portuguese/Brazilian strain (BAA43) | HA-MRSA, ST239, CC8            | Ref. 24     |
| Chinese strain (HS-522)      | HA-MRSA, ST239, CC8                      | Ref. 24     |
| USA400 (MW2)                 | CA-MRSA, ST1                             | Ref. 37     |
| Newman                       | MSSA isolate, ST8,                        | Ref. 38     |
| M SSA 476                    | MSSA isolate, ST1                        | Ref. 39     |
| 8325                         | MSSA, ST8                                | Ref. 40     |
| RF122                        | Bovine isolate                           | Ref. 41     |
| RN4220                       | Prophage/plasmid-cured, restriction-deficient derivative of 8325 | Ref. 40     |
| S. epidermidis               |                                          |             |
| 1457                         | Biofilm-forming clinical isolate          | Ref. 42     |
| ATCC12228                    | Non-biofilm forming skin isolate         | ATCC        |
| Other microorganisms         |                                          |             |
| Streptococcus pyogenes       | Serotype M3                               | F. DeLeo    |
| Micrococcus luteus ATCC9341  |                                          | ATCC        |
| Burkholderia cepacia 4A      |                                          |             |
| Escherichia coli DH5a        | Cloning strain                            | Invitrogen  |
| Candida albicans NIH 3172    |                                          |             |
KOdownRev, and admKOupFor and admKOupRev, respectively (Table 2). The obtained PCR products were cloned up and downstream of a spectinomycin resistance cassette that had previously been cloned in plasmid pBT2 via BamHI and Sall sites (29), using the restriction sites KpnI and BamHI, and Sall and HindIII, respectively. To facilitate plasmid excision after recombination, the secY antisense cassette from plasmid pKOR1 (26) was amplified using primers secYtetFor and secYtetRev (Table 2), introducing EcoRV sites, and cloned into the EcoRV site of the pBT construct. The final construct was transformed into strain USA300 (LAC) via strain RN4220 and the allelic replacement procedure was performed as described (26).

**Purification of Antimicrobial Activity**—Bacteria were grown in 50 ml TSB at 37 °C for 16 h, after which cultures were centrifuged and 30-ml aliquots of supernatants were collected and mixed with 10 ml of 1-butanol. Extraction was performed by shaking solutions in 37 °C for 2 h. After brief centrifugation, upper (butanol) phases were collected and concentrated by vacuum centrifugation. Dried samples were dissolved in 500 μl of 1 M urea and used for further procedures. 100 μl of those samples were applied onto a μRPC C2/C18 ST 4.6/100 column (GE Healthcare) and run on an HPLC/MS system (1100 HPLC system connected to an MSD Trap SL mass spectrometer, Agilent Technologies, Palo Alto, CA). An acetonitrile gradient from eluent A (0.1% TFA in water) to eluent B (0.1% TFA in acetonitrile) was applied as follows; 0% eluent B from 0 to 4 min, 0 to 50% eluent B linear gradient from 4 to 72 min, 50 to 100% eluent B linear gradient from 72 to 82 min, 100% eluent B from 82 to 90 min, 0% eluent B from 90 to 100 min. The flow rate was 0.5 ml/min and MS parameters were as published previously (17).

For the purification of antimicrobial peptides, strain SF8300 (USA300) was grown in 1 liter of TSB at 37 °C overnight. The supernatant was collected, acidified to pH 6 with HCl, and applied onto an HR16/20 column packed with cation exchange SOURCE 30S material (GE Healthcare) using an AKTA Purifier system (GE Healthcare). Eluent A (20 mM H₂PO₄ in water:acetonitrile (7:3, v/v)) and eluent B (1 M NaCl, 20 mM H₂PO₄ in water:acetonitrile (7:3, v/v)) were used to elute samples with a flow rate of 5 ml/min and a linear gradient (eluent B, 0 to 100% in 80 min). After agar diffusion assays on *Micrococcus luteus*, fractions with antimicrobial activity were collected and concentrated by lyophilization. The concentrated fractions were injected onto a ZORBAX SB-C18 9.4 × 25 cm column (Agilent) and eluted with a flow rate of 4 ml/min and a linear gradient of 0.1% TFA in acetonitrile:water (0 to 100% in 80 min) with fractionation. Fractions were concentrated by lyophilization and applied to the agar diffusion assay. Active fractions were collected and applied onto a SOURCE 5RPC ST 4.6/150 column (GE Healthcare). Samples were eluted using a 0.1% TFA acetonitrile:water gradient (0 to 20% in 2.5 min, 20 to 80% in 2.5 to 52.5 min, and 80 to 100% in 52.5 to 55 min) with a flow rate of 1 ml/min. Fractions were assayed for antimicrobial activity by the agar diffusion assay and analyzed by reversed phase (RP)-HPLC/electrospray ionization (ESI)-MS.

**Agar Diffusion Assay**—To determine antimicrobial activities of LC fractions or culture filtrates, *M. luteus* was used as a test strain. 100 μl of an overnight culture of *M. luteus* was added to 500 ml of autoclaved agar solution (5 g of casein hydrolysate peptone; 2.5 g of yeast extract; 2.5 g of NaCl; 1.31 g of K₂HPO₄; and 7.5 g of agar). 20–60 μl of culture filtrate or LC samples were applied on paper disks and placed onto the agar plates after drying. Plates were incubated at 37 °C overnight and examined for halo production around paper disks representing antimicrobial activity. To determine antimicrobial activities against *S. pyogenes*, the same procedure was used, but bacteria were added to Todd-Hewitt broth containing 0.2% yeast extract (THY).

**Determination of Minimal Bactericidal Concentration (MBC)**—Overnight cultures of each strain grown in proper media (Staphylococcal strains in TSB, Streptococcus pyogenes in THY, *M. luteus* in Luria-Bertani (LB) medium) were inoculated into appropriate media (20% TSB, 10% fetal bovine serum, 1 mM NaH₂PO₄, 150 mM NaCl, 50 mM NaHCO₃ for *S. aureus* LAC, RN4220 and *S. epidermidis* 1457 strains; TSB for *S. epidermidis* 12228; THY for *S. pyogenes*; LB for *M. luteus*) to achieve 2 × 10⁷ cfu/ml and 90–12228; THY for *S. pyogenes*; LB for *M. luteus*) to achieve 2 × 10⁷ cfu/ml and 90-% aliquots were dispensed in microtiter plate wells. GSM stock solutions (640, 320, 160, 80, 40, 20, and 10 μM) were prepared by serial dilutions with PBS. 10 μl of PSM serial stock dilutions were added into each well, and plates were incubated at 37 °C overnight. Cultures from the wells with no visible growth were spread on agar plates, and cfus were counted after overnight incubation at 37 °C. MBC was determined as the lowest concentration of PSM, at which >99% of inoculated cells were killed.

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**TABLE 2**

| Oligonucleotide               | Sequence                                      |
|-------------------------------|-----------------------------------------------|
| **Deletion of psmA operon**   |                                               |
| psmAfor1                      | GGGGACAAAGTTTGTACAAAAAGACGGCTTGTGGACACGCCCTGCCAG |
| psmArev1                      | GATCCCAGGATGATAACCCATTAACATTTTCTCC |
| psmAfor2                      | GGGGACACCTTTTTACAGAAAGCTTGOTGACGACTATGCGCACAG |
| psmArev2                      | GCGAATTGAACTTTAAAAATTCCTGAGCCACTATAC |
| **Deletion of adm cluster**   |                                               |
| admKOdownFor                  | A/TGCGGATCCGGATGTTGCGCTTAAACGCTATAAAAG |
| admKOdownRev                  | A/TGCGGATCCGGATGTTGCGCTTAAACGCTATAAAAG |
| admKOupFor                    | TGGCAGTCAGCAGTGATGACAGAAAGACGGAGAGCTG|
| admKOupRev                    | TGGCAGTCAGCAGTGATGACAGAAAGACGGAGAGCTG |
| **Cloning of secY cassette**  |                                               |
| secYtetFor                    | GATCCCCCGGTATGAAAAAGAGATAGAAAG |
| secYtetRev                    | GATCCCCCGGTCTGTTTCTTCATAGAAAT |

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**MSM Antimicrobial Activities**
FIGURE 1. Analysis of antimicrobial activity in *S. aureus* strains. *A*, dried 1-butanol extracts (corresponding to 1.2 ml of culture) of various *S. aureus* strains were dissolved in 8 M urea, applied on filter disks, and placed on *M. luteus* test plates. Test plates were grown for 24 h. *B*, peak areas corresponding to the m/z 1046.1 peak at 66 min, obtained by integration of extracted ion chromatograms of m/z 1046.1. 100-μl samples of 1-butanol extracts (corresponding to 6 ml of extracted culture) were applied to a µRPC C2/C18 ST 4.6 × 100 mm column and a gradient from 0.1% TFA/water to 0.1% TFA/acetonitrile was run at 0.5 ml/min. Example chromatograms are shown in *C*. The largest peak found in the USA300 strain is marked by an arrow. *D*, mass chromatogram of the peak at ~66 min in strain USA300.
RESULTS

*S. aureus* Culture Filtrates Contain Peptides Producing m/z Peaks during HPLC/MS Analysis Indicative of Adm2 Production—To examine whether *S. aureus* strains produce the *S. aureus* lantibiotics Adm1 or Adm2, we investigated six strains representative of the major MRSA CC8 subclones (24), all of which contain the *adm* system, and all *S. aureus* strains, whose genomes have been sequenced and contain the genetic information to produce the Adm lantibiotics. We extracted stationary growth phase culture filtrates with 1-butanol, a procedure known to enrich lantibiotics of the epidermin/gallidermin family (30), determined antimicrobial activities on *M. luteus* agar plates (Fig. 1A) and applied the dried and redissolved butanol extracts to a µRPC C2/C18 ST 4.6/100 (GE Healthcare) reversed phase HPLC column. The column was run on an HPLC system coupled to an Ion Trap mass spectrometer with ESI. We analyzed the entire elution range (0–100% acetonitrile) for m/z values corresponding to those expected for Adm1 and Adm2. In the elution ion chromatograms of several strains, we detected peaks with m/z 1046.1 ((Adm2 + 2H+)/2) at elution times of ~66 min (Fig. 1, B and C). The m/z 1046.1 peak was accompanied by the corresponding triple charged ion of m/z 698.2 ((Adm2 + 3H+)/3), indicative of a peptide with a calculated mass of 2090.1 Da (Fig. 1D). This mass is in very good agreement with the theoretical mass of Adm2 of 2091 Da and the experimental 2089.5 Da value determined by Daly et al. (13).

Of note, production of an antimicrobial substance as determined by the agar diffusion assay (Fig. 1A) correlated with presence of an m/z 1046.1 peak at ~66 min. Additional, minor peaks of m/z 1046.1 found in some strains at other retention times were not accompanied by the corresponding triple-charged ion and are thus not related to a peptide with a mass of 2090 Da. Furthermore, we did not find ions related to the theoretical Adm1 peptide, in agreement with the observations by Daly et al. (13). These results indicated that the antimicrobial activity corresponding to a peptide of ~2090 Da eluting at ~66 min represents the major antimicrobial activity in the *S. aureus* extracts and, at first, suggested that it is the Adm2 lantibiotic.

Antimicrobial Activity in *S. aureus* Culture Filtrates Is Due to PSMs—As an alternative hypothesis, we examined whether the antimicrobial activity may be due to production of PSMs. To test whether PSMs may be involved in the antimicrobial activity of *S. aureus* strains, we tested 1-butanol extracts of *S. aureus* USA300 (LAC) wild-type and isogenic *psm* mutants on *M. luteus* and *S. pyogenes* test plates (Fig. 2A) and by RP-HPLC/ESI-MS (Fig. 2B). All PSM peptides could be extracted using 1-butanol. In addition to the *psma*, *psmB*, and *hld* mutants from a previous study (17), we constructed *psma* double mutants (*psma/psmB*, *psma/hld*) and a triple mutant (*psma/psmB/hld*). In the latter, all known *S. aureus* *psm* genes are deleted or, in the case of *hld*, expression is inactivated by mutation of the start codon. Moreover, we tested an *agr* mutant, in which no PSM peptides are produced owing to strict regulation of PSMs by the *agr* quorum-sensing system (17, 25). Finally, we constructed a deletion mutant of the *adm* locus in USA300 to analyze whether the antimicrobial activity of that strain is due to the production of the Adm lantibiotic.

The results clearly demonstrated that the antimicrobial activity of strain USA300 is due to PSMs rather than the Adm2 bacteriocin. First, antimicrobial activities were significantly reduced in several *psm* deletion mutants and completely absent in the *psma/psmB/hld* deletion strain, whereas there was no significant difference compared with the wild-type for the *adm* deletion mutant. Second, RP-HPLC/ESI-MS analysis showed that presence of the peak indicating production of the 2090-Da peptide was abolished in the *psma/psmB/hld* deletion but not affected in the *adm* deletion strain. Furthermore, this peak was found predominantly in strains known to produce high amounts of PSMs, such as USA300, USA400, and USA500 (17, 24) (Fig. 1, B and C), in further support of the idea that the corresponding peptide is PSM-derived.
The results obtained in the agar diffusion assay with the different psm mutant strains indicated that primarily the PSM\(\alpha\) peptides and the \(\delta\)-toxin (Hld) may be the source(s) of the antimicrobial activity (Fig. 2A). RP-HPLC/ESI-MS analysis showed that the \(m/z\) 1046.1 peak at \(\sim 66\) min corresponding to the 2090-Da peptide was only observed in extracts from strains that contained the \(psm\) genes, but not those in which these genes were deleted (Fig. 2B). These results indicated that the antimicrobial activity in strain USA300 is linked predominantly to PSM\(\alpha\) peptides.

\textit{S. aureus} Antimicrobial Activity Is Due to Proteolytically Processed PSM\(\alpha 1\) and PSM\(\alpha 2\) Peptides—To further confirm this hypothesis, we purified the antimicrobial activity using 1-butanol extraction followed by a series of preparative chromatographic steps, including cation exchange and reversed-phase chromatography. During purification, fractions were analyzed for antimicrobial activity on \(M. luteus\) plates. In the last chromatographic step, antimicrobial activity correlated with two peptides with calculated masses of 2090 and 2072 Da (Fig. 3).

N-terminal sequencing of the peptides demonstrated that these peptides were N-terminally processed products of the PSM\(\alpha 1\) and PSM\(\alpha 2\) peptides, in which the two N-terminal amino acids were missing (Fig. 4).

Taken together, our results demonstrated that the antimicrobial activity of USA300 is due to proteolytically processed PSM\(\alpha 1\) and PSM\(\alpha 2\) peptides rather than the Adm2 lantibiotic. Culture filtrates of all strains that showed antimicrobial activity produced ions corresponding to the peptides with masses of
TABLE 3

Minimal bactericidal concentrations of S. aureus PSMs

| PSM          | S. aureus LAC (KAM<sup>a</sup>) | S. aureus RN4220 (KAM<sup>a</sup>) | S. epidermidis 145<sup>b</sup> (KAM<sup>a</sup>) | S. epidermidis 12228 (TSB<sup>b</sup>) | S. pyogenes (THY<sup>b</sup>) | M. luteus (LB<sup>b</sup>) |
|--------------|---------------------------------|-----------------------------------|-----------------------------------------------|-------------------------------------|----------------------------|----------------------------|
| a1           | >64                             | >64                               | >64                                           | >64                                 | >64                        | >64                        |
| a2           | >64                             | >64                               | >64                                           | >64                                 | >64                        | >64                        |
| a<sub>3</sub>, a<sub>4</sub>, β1, β2, δ-toxin, mec<sup>a</sup> | >64                             | >64                               | >64                                           | >64                                 | >64                        | >64                        |
| a1Δ1-2       | >64                             | >64                               | >64                                           | >64                                 | >64                        | >64                        |
| a2Δ1-2       | >64                             | >64                               | >64                                           | >64                                 | >64                        | >64                        |

<sup>a</sup> Medium used in MBC assays.

<sup>b</sup> PSM-mec is present in some MRSA and methicillin-resistant S. epidermidis.

2090 and 2072 Da during HPLC with similar retention times, indicating that the processed PSMa1 and PSMa2 peptides are responsible for antimicrobial activity in all those tested S. aureus strains. Remarkably, the mass of the processed PSMa1 peptide corresponds exactly to the mass of Adm2. This coincidence explains the previous misinterpretation of the antimicrobial activity of USA300 and other S. aureus strains as being Adm2-derived (13).

**Antimicrobial Activity among S. aureus PSMs Is Limited to Processed PSMa1 and PSMa2**—To further investigate antimicrobial activities of S. aureus PSM peptides, including the two processed PSMa peptides, we analyzed antimicrobial activities of pure, synthetic peptides corresponding to all known PSM peptides of S. aureus. To that end, we first applied 15 μl of 10 mg/ml solutions on filters and placed them on test plates containing different bacteria. This concentration exceeds the maximal concentrations of PSMs detected in bacterial culture filtrates by at least one log (16–18). On S. pyogenes and M. luteus test plates, the two processed S. aureus PSMa peptides (PSMa1Δ1-2, PSMa2Δ1-2) showed considerable antimicrobial activity, in contrast to the integral PSMa1 and PSMa2 peptides and all other tested PSMs (data not shown). No activities of PSMs were detected on Burkholderia cepacia, Escherichia coli, Candida albicans, S. aureus, and S. epidermidis plates (data not shown). Furthermore, we determined MBCs of all S. aureus PSM peptides (Table 3). Provided that strains exhibited growth in that medium, we used a special carbonate-containing growth medium (killing assay medium), in which antimicrobial peptides were previously shown to develop more pronounced activity (31). In addition to activities against M. luteus, we determined those against several S. aureus and S. epidermidis strains, and S. pyogenes. Unprocessed PSM peptides of S. aureus did not elicit antimicrobial activities against any of the tested bacteria, except for very low activities of PSMa1 and PSMa2 against S. pyogenes. MBCs of S. aureus PSMa1Δ1-2 and PSMa2Δ1-2 were strongly increased compared with PSMa1 and PSMa2, in particular when tested against S. pyogenes. In summary, our experiments demonstrated significant antimicrobial activities of the PSMa1Δ1-2 and PSMa2Δ1-2 peptides, particularly against S. pyogenes. In contrast, unprocessed PSMs exhibited no, or in rare cases, weak antimicrobial activities.

**DISCUSSION**

Exceptional capacity to colonize humans is a frequently discussed possible reason for the epidemiological success of CA-MRSA strains, such as USA300, as pathogens (32, 33). Although there is no evidence to support this hypothesis, which is mainly due to the lack of appropriate colonization models, two molecular factors have been suggested to potentially promote colonization of USA300. First, most USA300 strains contain the arginine catabolic mobile element, whose arginine deiminase operon has been implied in promoting colonization mainly via an impact on pH homeostasis (32, 34). This element is absent from most other S. aureus. Second, the Adm2 lantibiotic has been reported to be expressed strongly in strains of the ST8 and ST80 sequence types, which contain the epidemic U.S. and European CA-MRSA clones, respectively. On those grounds, Adm2 was speculated to contribute to the spread of those strains by a competitive advantage over other bacteria, including staphylococci (13).

In the present study, we investigated the molecular basis of the antimicrobial activity found in USA300 and other S. aureus strains to evaluate whether it is due to the Adm2 lantibiotic, as proposed previously (13). Our results indicate that antimicrobial activities of S. aureus strains are based on PSM peptides rather than lantibiotic-type bacteriocin production. Although it has been unequivocally shown that the adm operon codes for a functional lantibiotic (13), previous conclusions based on mass spectrometric analysis suggesting presence of the Adm2 lantibiotic in S. aureus culture filtrates may have been incorrect, owing to the coincidentally equal masses of the antimicrobially active, processed PSMa1 peptide, PSMa1Δ1-2, and Adm2. Thus, the biological function of the adm operons present in many S. aureus strains remains unresolved. It is possible that the Adm lantibiotic(s) are only produced under specific in vivo conditions that have not been or cannot be reproduced in the laboratory.

Among S. aureus PSMs, significant antimicrobial activity was detected mostly in proteolytically processed PSMs, suggesting that PSM-based bacterial interference may play a role in microenvironments with proteolytic activity or under conditions with strong production of S. aureus exoproteases. Except for weak activity of PSMa2Δ1-2 against S. aureus RN4220 and S. epidermidis ATCC12228, we did not find considerable activity of any PSM or PSM derivative against S. aureus or S. epidermidis strains, indicating that the antimicrobial properties of S. aureus PSMs are likely not involved in a significant competitive ecological advantage within the species or genus. Contrarily, the pathogen and epithelial colonizer S. pyogenes was highly sensitive to PSM antimicrobial activity, suggesting that PSMs may have a biological function in bacterial interference of S. aureus with that competing bacterium. This finding is of particular interest, as staphylococci and streptococci are niche
competitors in the human body and are not commonly found to co-colonize. Although mechanisms related to hydrogen peroxide resistance have been proposed to cause an advantage for streptococci in the nasopharynx (35), mechanisms contributing to predominantly staphylococcal colonization of the nose are not understood. Our findings suggest that PSM-based bacterial interference may represent such a mechanism; strong production of PSMs in CA-MRSA may thus contribute to the exceptional capacity of those strains to colonize and spread in the population.

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