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Clinical MRSA isolates from skin and soft tissue infections show increased *in vitro* production of phenol soluble modulins

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Summary

Background—Phenol-soluble modulins (PSMs) are amphipathic, pro-inflammatory proteins secreted by most *Staphylococcus aureus* isolates. This study tested the hypothesis that *in vitro* PSM production levels are associated with specific clinical phenotypes.

Methods—177 methicillin-resistant *S. aureus* (MRSA) isolates from infective endocarditis (IE), skin and soft tissue infection (SSTI), and hospital-acquired/ventilator-associated pneumonia (HAP) were matched by geographic origin, then genotyped using *spa*-typing. *In vitro* PSM production was measured by high performance liquid chromatography/mass spectrometry. Statistical analysis was performed using Chi-squared or Kruskal–Wallis tests as appropriate.

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Conferences where the work was presented

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Conflicts of interest

Mr. Berlon reports grants from IDSA Medical Scholars Program, grants from Duke Travel Grant, during the conduct of the study. Dr. Woods reports other from bio-Merieux, non-financial support and other from Becton Dickinson, grants from Novartis, outside the submitted work. Dr. Otto reports grants from NIAID, during the conduct of the study; in addition, Dr. Otto has a patent 8,211,445 issued. Dr. Fowler reports grants from National Institutes of Health (K24-AI093969 and R01-AI068804), during the conduct of the study; personal fees from Board Membership Merck, personal fees from Consultancy from Pfizer, Novartis, Galderma, Novadigm, Durata, Debiopharm, Genentech, Achaogen, Affinium, Medicines Co., Cerexa, Tetrphase, Trius, MedImmune, Bayer, Theravance, Cubist, Basilea, Affinergy; personal fees from Duke University Medical Center, grants from Institutional Grants/grants pending NIH, MedImmune, Forest/Cerexa, Pfizer, Merck, Advanced Liquid Logics, Theravance, Novartis, and Cubist, personal fees from Royalties (UpTo-Date), personal fees from Payment for development of educational presentations (Green Cross, Cubist, Cerexa, Durata, Theravance), outside the submitted work; In addition, Dr. Fowler has a patent NCGR pending.

Results—*Spa* type 1 was significantly more common in SSTI isolates (62.7% SSTI; 1.7% IE; 16.9% HAP; $p < 0.0001$) while HAP and IE isolates were more commonly *spa* type 2 (0% SSTI; 37.3% IE; 40.7% HAP; $p < 0.0001$). USA300 isolates produced the highest levels of PSMs *in vitro*. SSTI isolates produced significantly higher quantities of PSM α 1-4, PSM β 1, and δ -toxin than other isolates ($p < 0.001$). These findings persisted when USA300 isolates were excluded from analysis.

Keywords

Phenol soluble modulins; MRSA; Skin and soft tissue infection; Pneumonia; Endocarditis

Introduction

Staphylococcus aureus causes a diverse array of human infections including infective endocarditis (IE),¹ skin and soft tissue infection (SSTI),² and hospital-acquired/ventilator-acquired pneumonia (HAP).³ Although the repertoire of virulence factors produced by a particular clone of *S. aureus* is thought to influence the type of infection that it causes, this association is poorly understood.

Phenol-soluble modulins (PSMs) are small, amphipathic proteins that contribute to bacterial pathogenesis^{4,5} and are widely present in *S. aureus*.⁶ Eight such proteins have been identified in *S. aureus*: six are 20–25 amino acid long α -types (PSM α 1-4, δ -toxin, PSM-mec) and two are 44 amino acid long β -types (PSM β 1, PSM β 2).⁵ PSM α peptides are co-transcribed from the *psm α* and PSM β peptides from the *psm β* locus.³² The δ -toxin (gene *hld*) is encoded within RNIII, the regulatory molecule of the accessory gene regulator (*agr*) locus. All PSMs are under strict and direct regulation by *agr*.³² PSM peptides are produced by all *S. aureus* strains (except naturally occurring *agr* mutants) due to location of the encoding genes on the core genome or on widely distributed pathogenicity islands.⁶ The SCCmec-encoded PSM-mec is found on specific SCCmec elements (types II, III, and VIII) and is also *agr*-regulated, although the precise mechanism of *agr* regulation of *psm-mec* expression is unknown.^{36,37} Owing to co-transcription, the values of different PSM α peptides, as well as those of the PSM β peptides, are closely linked to each other, although differential proteolytic processing or export may theoretically lead to differences of concentration of secreted peptide. Furthermore, regulation of all PSMs by *agr* is usually reflected by a relatively constant shift of concentrations of all PSM peptides under different conditions.

A growing body of evidence from both *in vitro* and *in vivo* studies suggests that PSMs are important in the development of skin and soft tissue infections (SSTI). PSMs and their proteolytic products facilitate *S. aureus* colonization⁷ and dispersion⁸ on skin. In animal studies, PSM deletion strains of *S. aureus* cause smaller SSTI lesions in both mice⁹ and rabbits¹⁰ relative to isogenic wild type strains. PSM production is also significantly higher in USA300, a community-associated methicillin-resistant *S. aureus* (CA-MRSA) strain strongly associated with SSTI in the United States,¹¹ than other variants of MRSA.^{9,12}

Based on these observations, we hypothesized that MRSA isolates obtained from SSTIs produce higher levels of PSMs than isolates associated with other infection types. To test

this hypothesis, we utilized a unique resource of well-characterized international MRSA isolates from multiple infection sites including SSTI,^{13,14} HAP,¹⁵ and IE.^{16,17}

Materials and methods

S. aureus isolates

IE isolates were obtained from the Microbiologic Repository of the International Collaboration on Endocarditis – Prospective Cohort Study (ICE-PCS).¹⁶ The repository contains >1600 isolates collected prospectively between June 2000 and September 2006 from patients with definite or possible IE at 24 sites in 12 countries.¹⁸ Patients met all of the following inclusion criteria: prospective identification at a center with a minimum of 12 cases per year and access to cardiac surgery, adequate records to complete a 275 item standard case report form, and evaluation at time of initial presentation.¹⁷ Bacterial specimens were collected from all patients through blood cultures.

SSTI isolates were obtained from a repository created as part of the Assessment of Telavancin in cSSSI (ATLAS) trials, two methodologically identical, randomized, multinational, parallel-group active-controlled phase III clinical trials including 2079 patients from 129 centers in 21 countries.¹³ Patients met all of the following inclusion criteria: male or non-pregnant female aged ≥ 18 years old, diagnosis of complicated SSTI caused by a suspected or confirmed gram-positive organism, and need for ≥ 7 days of parenteral antibiotic therapy. Bacterial specimens were collected from all patients through needle aspiration or, if necessary, surgical procedures.^{13,14}

HAP isolates were obtained from a repository created as part of the Assessment of Telavancin for Hospital-Acquired Pneumonia (ATTAIN) trials,¹⁵ two methodologically identical, randomized, multinational, parallel-group double-blinded phase III clinical trials including 1532 patients from 274 centers in 38 countries. Patients met both of the following inclusion criteria: male or non-pregnant female aged ≥ 18 years old, and diagnosis of pneumonia acquired after 48 h in an inpatient, acute, or chronic care facility or which developed within 1 week of discharge from one of these facilities. Bacterial specimens were collected from all patients through blood cultures and respiratory samples.

Definitions

IE was defined according to the modified Duke criteria.¹⁸ SSTI was defined by the presence of at least one of the following: cellulitis, abscess requiring surgical drainage, infected wound or ulcer, or infected burn. In addition, purulent drainage, fluid collection, or ≥ 3 of the following signs and symptoms were required: erythema; heat and/or localized warmth, fluctuance, swelling and/or induration, pain and/or tenderness to palpation, fever with temperature >38 °C, or WBC count >10,000 cells/mm³ or >15% bands.¹³ HAP was defined as identification of an organism consistent with a respiratory pathogen in respiratory tract or blood, or as the presence of ≥ 2 of the following: cough, purulent sputum, auscultatory findings, dyspnea, tachypnea, or hypoxemia. Patients were additionally required to have ≥ 2 of the following: temperature >38 °C or <35 °C, respiratory rate >30 breaths/min, pulse >120 beats/min, altered mental status, need for mechanical ventilation, or white blood cell

count >10,000 cells/mm³, <4500 cells/mm³, or >15% bands. All patients were required to have new or progressive infiltrates, consolidation, and an adequate respiratory specimen for Gram stain and culture.¹⁵ All MRSA isolates meeting these definitions were considered for inclusion in this study.

USA300 strains were defined by the presence of all of the following: 1) *spa* type 1 or inferred genotype, 2) presence of the genetic elements for both Panton Valentine Leukocidin (PVL) and Arginine Catabolic Mobile Element (ACME) by PCR, and 3) confirmatory USA300 genotype by pulsed field gel electrophoresis (PFGE) in all SSTI isolates.¹⁹

Matching methods

Each clinical group (IE, HAP, and SSTI) was sorted and matched 1:1:1 by continent with the total number included from each being dictated by the clinical group with the fewest isolates arising from that continent. In the event that one or both remaining studies had isolates in excess of this amount, inclusion was determined by taking every Xth isolate such that the totals selected for each continent were all equivalent, with X being defined as (number of isolates in larger study)/(number of isolates in smallest study).

Growth and supernatant collection

Trypticase soy agar (TSA) plates were prepared, inoculated with specimens frozen in tryptic soy broth (TSB)/glycerol stock, and incubated overnight at 37 °C. Approximately 1 µL of this bacteria was inoculated into 10 mL TSB in 50 mL conical tubes and grown overnight at 37 °C and 200 RPM. The OD₆₀₀ of each sample was then measured, and an amount of bacteria necessary to reach a calculated starting OD₆₀₀ of 0.1 was added to new 50 mL conical tubes containing 10 mL TSB. These were grown for precisely 8 h at 37 °C and 200 RPM, then immediately placed on ice to arrest growth. Samples were centrifuged at 4 °C and 4000 RPM for 10 min at which time 9 mL of supernatant were collected from each and stored at -80 °C until further use.

High-performance liquid chromatography (HPLC)/Mass spectrometry

Quantitation of PSMs in the supernatant was performed using reversed phase HPLC/ electrospray ionization mass spectrometry (RP-HPLC/ESI-MS) as described previously⁹ by investigators who were blinded to the clinical source of the isolate. Analysis of the same supernatant was repeated and the two runs averaged to obtain final data for each isolate and PSM subtype.

Spa genotyping

TSA plates were inoculated with specimens frozen in TSB/glycerol stock and incubated overnight at 37 °C. Bacteria was harvested from the plates and then resuspended in 300 µL of Microbead solution containing 20 µL of lysostaphin. DNA was then extracted using the MoBio Ultraclean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. PCR was performed using TIGR-F and TIGR-R primers as described previously²⁰ before submission to the Duke University DNA sequencing facility to obtain forward and reverse sequences. Approximately 10% of isolates selected at random underwent additional confirmatory *spa* typing for quality control

purposes, with >90% of these matching in one run, and all matching after two confirmatory runs. In the event of poor sequences or discrepancies, isolates were re-cultured and the process repeated until a consensus was reached.

Spa type nomenclature

Spa types were assigned using the eGenomics online platform (<http://tools.egenomics.com>). Genotypes defined by the Ridom platform (<http://www.ridom.de/>), an alternative *spa* genotyping schema, were also provided for the most common identified strains encountered in the study.

Detection of ACME and PVL

Uniplex PCR was used to identify presence of *arcA* for ACME and *lukS/F* for PVL in all possible CC8 isolates with *S. aureus* genus-specific 16S rRNA serving as an internal control. *S. aureus* genomic DNA was extracted as described above. Two microliters of DNA from each isolate was PCR amplified under the following conditions using Platinum® Taq DNA Polymerase (Invitrogen, Grand Island, NY) and 0.2 µM of each forward and reverse primers [*arcA*-F 5'-GCAGCAGAATCTATTACTGAGCC-3' and *arcA*-R 5'-TGCTAACTTTTCTATTGCTTGAGC-3'²¹; *luk*-PV-1 5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3' and *luk*-PV-2 5'-GCATCAAGTGTATTGGATAGCAAAAGC-3'²²; and 16S rRNA-F 5'-AACTCTGTTATTAGGGAAGAACA-3' and 16S rRNA-R 5'-CCACCTTCCTCCGGTTTGTACC-3'²³]; an initial denaturation step of 95 °C for 5 min, 35 cycles of 95 °C for 60 s, 55 °C for 30 s and 72 °C for 60 s followed by 72 °C for 10 min. The PCR amplicons (10 µl) were visualized using UV light after electrophoresis in a 1% agarose gel containing ethidium bromide.

PFGE

PFGE was performed on all SSTI isolates.²⁴ PFGE banding patterns were analyzed with appropriate controls using Bio-Numerics software (Applied Maths, Kortrijk, Belgium) and defined as USA300 using standard definitions.¹⁹

Statistical methods

Simple descriptive statistics were used for bacterial production profiles with means presented for continuous variables. Differences in *spa* type distribution were measured with χ^2 analysis. PSM production was quantitated and averaged across two runs of the same samples. Statistical differences in mean PSM production between groups were evaluated via separate Kruskal–Wallis tests for each peptide. In all cases a p value 0.05 was considered statistically significant.

This study was approved by the Duke University Medical Center Institutional Review Board.

Results

A total of 177 MRSA IE, SSTI, and HAP isolates forming 59 geographically-matched triplets were included in the analysis. Of the 59 triplets, 37 (62.7%) came from the United States, 16 (27.1%) came from Europe, and 6 (10.2%) came from Australia.

Patient characteristics

Patients with SSTI tended to be younger on average (45.2 years (SSTI) vs 63.2 years (IE) vs 65.4 years (HAP); $p < 0.0001$), but there was no significant difference in gender among clinical groups. Other illness-specific parameters for SSTI, HAP, and IE are presented in Tables 1–3, respectively.

Spa genotyping

A total of 38 known and 5 new *spa* types were identified among the 177 isolates. Of these, the 5 most common *spa* types (1, 2, 3, 16, and 388 [Ridom nomenclature t008, t002, t037, t018, and t041, respectively]) accounted for 67.8% of the total collection (Fig. 1). The distribution of *spa* types differed significantly in the three clinical groups. *Spa* type 1 (Ridom t008) was significantly more common in the SSTI group (SSTI 38/59 [62.7%] vs HAP 10/59 [16.9%] vs IE 1/59 [1.7%]; $p < 0.0001$), while *spa* type 2 (Ridom t002) was more common in the HAP and IE groups (SSTI 0/59 [0%] vs HAP 24/59 [37.3%] vs IE 22/59 [40.7%], $p < 0.0001$).

Levels of *in vitro* PSM production

Levels of PSM α 1, PSM α 2, PSM α 3, PSM α 4, PSM β 1, and δ -toxin were significantly higher among SSTI isolates than either IE or HAP isolates (p values < 0.0001 for all; Fig. 2). By contrast, levels of PSM-mec were significantly lower among SSTI isolates than the other two clinical groups ($p < 0.0001$). Finally, levels of PSM β 2 were significantly lower in HAP isolates with relatively similar production in both IE and SSTI isolates ($p = 0.0008$). Aggregate analysis of *spa* types 1 and 2 revealed a strikingly similar pattern, with levels of PSM α 1, PSM α 2, PSM α 3, PSM α 4, PSM β 1, and δ -toxin being higher among *spa* type 1 isolates, and levels of PSM-mec being higher among *spa* type 2 isolates (p values < 0.0001 for all).

Levels of *in vitro* PSM production for USA300 isolates

USA300 isolates were associated with significantly higher levels of PSM α 1, PSM α 2, PSM α 3, PSM α 4, PSM β 1, PSM β 2, and δ -toxin than non USA300 isolates ($p < 0.002$ for all) and lower production of PSM-mec than non USA300 isolates ($p = 0.0024$) (Fig. 3). Even within the SSTI isolates, USA300 isolates exhibited significantly higher mean levels of PSM α 2, PSM α 3, PSM α 4, and δ -toxin (p values < 0.02 ; data not shown).

To ensure that the association between SSTI infection and higher PSM levels was not due to confounding introduced by a higher prevalence of USA300 in the SSTI group, we excluded USA300 isolates and repeated the analysis to compare mean levels of *in vitro* PSM production in the three clinical groups. Even after exclusion of USA300 isolates, SSTI isolates continued to be associated with significantly higher levels of PSM α 3, PSM α 4,

PSM β 1 and δ -toxin and lower production of PSM-mec than IE or HAP isolates ($p = .05$ for all) (Fig. 4).

Associations with clinical outcome of infection and levels of *in vitro* PSM production

No significant associations were identified between *in vitro* PSM levels and clinical outcomes (Supplemental Table 1; data not shown).

Discussion

In this study, we evaluated associations between *in vitro* PSM production and specific types of infection. Our results were striking: *in vitro* PSM levels were significantly higher in MRSA isolates obtained from patients with SSTI than MRSA isolates from patients with either HAP or IE. This finding persisted even after USA300 isolates were removed from the analysis. These results provide strong evidence for an association between level of PSM production and the type of infection caused by MRSA, which is of particular interest clinically because intracellular PSM accumulation disrupts *S. aureus* cellular division and plasma membrane integrity resulting in cell death.⁴⁴ As such, the targeting of individual PSMs or the PSM transporter Pmt have been proposed as novel avenues for anti-staphylococcal drug development.

The association between PSM production and SSTI is consistent with our understanding of SSTI pathophysiology. In previous studies, PSM subtypes α 1-4 and δ -toxin promote the ability of *S. aureus* to spread across skin,⁸ reduce microbial competition,⁷ recruit, activate, and lyse neutrophils,⁹ and disrupt detection by the adaptive immune system by interfering with dendritic cell function.²⁵ Taken together, these findings provide potential biological plausibility for the higher levels of PSM that we observed in strains of *S. aureus* causing SSTI.

USA300 isolates in this study exhibited high levels of PSM, a finding that is consistent with previous reports.^{9,12} USA300 is now the predominant strain of *S. aureus* in the United States.²⁶ Although USA300 has been described in Europe,²⁷ Asia²⁸ and Australia,²⁹ it remains uncommon in those regions. Using a cohort of isolates from Europe, Australia, and the United States, we show that the relationship between PSM production and SSTI persists in the absence of USA300. This finding suggests that some or all of the currently identified PSM peptides are important components in the pathogenesis of staphylococcal SSTI.

Overall, HAP and IE isolates in this study produced significantly less PSM α 1-4 and δ -toxin. Although the role of PSMs in HAP and IE is still largely unknown, one potential explanation involves their role in biofilm homeostasis. Reduced production of PSMs leads to excessive biofilm formation with decreased capacity for systemic dissemination.⁴ Because biofilm is thought to play a key role in the pathogenesis of ventilator-associated pneumonia³⁰ and IE,³¹ it is possible that reduced production of PSMs contributes to the pathogenesis of patients with these particular infection types. On the other hand, PSMs are strictly regulated by quorum-sensing³² and serum lipoproteins found in human plasma have been shown to cause this mechanism to fail through ApoB1 sequestration of AIP.³³ Furthermore, PSM proteins themselves are neutralized by high low density lipoproteins at physiologic concentrations.³⁴

Thus, the endovascular nature of IE may ultimately reduce the importance of PSMs in the pathogenesis of this type of infection. In general, the role of PSMs in bloodstream infections awaits clarification. While production of a PSM α 3 variant with reduced cytolytic and pro-inflammatory potential is linked to more severe hematogenous seeding,³⁵ PSM knockout strains were associated with decreased mortality in a mouse septicemia model.⁹

Of note, HAP and IE isolates in this study produced significantly higher levels of PSM-mec. In contrast to the other PSM peptides, whose genes are generally found in all *S. aureus* isolates, this PSM peptide is encoded on SCCmec elements of types II, III, and VIII.^{36,37} Production of PSM-mec has been associated with increased virulence,³⁶ but it is also embedded in a regulatory RNA whose impact on virulence is opposite to that of the PSM-mec peptide.³⁸ The inverse relationship between the production of PSM α 1-4/ δ -toxin and PSM-mec in our study is interesting, particularly given its status as the only known PSM contained on a mobile genetic element³⁶ and the reported negative impact that the *psm-mec* regulatory RNA has on virulence in a murine skin infection model.³⁸ Virtually all *S. aureus* toxins including PSMs are under the control of the global accessory gene regulator (*agr*) locus.³⁹ *agr* is recognized as a quorum-sensing gene cluster that generally up-regulates production of secreted virulence factors and down-regulates production of cell-associated virulence factors in a growth phase dependent manner. Recently, it has been also suggested that *agr* plays the critical role in cellular metabolism.⁴⁰ The importance of *agr* locus has been well established in a variety of animal models of infection.⁴¹ Mechanistically, previous studies have demonstrated that the *psm-mec* RNA influences virulence, at least in part, by inhibiting the translation of *agrA*, a key positive regulator of all other PSM subtypes.^{32,38} Thus, high PSM-mec production should result in lower production of other PSMs, although it has been shown that this effect and the overall impact on virulence of the *psm-mec* locus is highly strain-dependent.^{37,42} Our results support such an inverse relationship between PSM-mec and other forms of PSMs. Although the regulatory pathways governing PSM production are incompletely understood, it is possible that regulatory mechanisms designed to influence PSM-mec production may contribute to the likelihood that an isolate will cause SSTI or HAP/IE through differential expression of all other PSM subtypes.

The role of PSM β peptides in the pathogenesis of *S. aureus* infection is less clear, and the majority of knockout studies to date have shown their absence to have little effect on observed metrics: PSM β peptides are barely cytolytic,⁹ and *in vitro* production of PSM β peptides in *S. aureus* is very low in contrast to several other staphylococci such as *Staphylococcus epidermidis*.⁴³ In our study, levels of PSM β 1 were highest in SSTI isolates while levels of PSM β 2 were significantly lower in HAP isolates. These results suggest that more work remains to be done to define the role of the PSM β peptides in the pathogenesis of *S. aureus* infection.

Our study has limitations. First, our comparisons were susceptible to confounding by bacterial genotype, and it is possible that the association we found is due to another factor entirely. To address this, we matched isolates on the basis of geographic origin and repeated our analyses after excluding the primary confounding clone, USA300. The fact that we reached the same conclusions both with and without USA300 isolates in the analysis supports the generalizability of these results. From the opposite perspective, the fact that

nearly all of the genotypes found within SSTI are either known to be high PSM producers or had relatively higher production compared to IE/HAP may indicate that increased PSM production is responsible for the skewed genotype distribution we observed. Further studies are necessary to distinguish between these two possibilities. Second, our adjustment for geographic variation was based on the continent of origin, which may be imprecise. Third, PSM levels were quantitated using *in vitro* assays. Thus, PSM levels produced by these isolates in a clinical infection may differ. Fourth, our analysis was limited to MRSA; current studies in methicillin susceptible *S. aureus* isolates are planned. Finally, our results only identify an association between *in vitro* PSM levels and clinical infection type. The biological basis of this finding needs to be established *in vivo*.

Despite these limitations, however, our study demonstrates that MRSA isolates from patients with SSTI produce significantly higher levels of PSM *in vitro* than geographically matched MRSA isolates from patients with either HAP or IE, even when USA300 was excluded from the analysis. This association may represent a fundamental role for PSMs in the pathogenesis of SSTI that is not present in other types of infection caused by MRSA. Further *in vivo* studies using SSTI models from globally diverse clonal backgrounds involving PSM knockout models are necessary to establish the importance of PSMs in the initial pathogenesis of infections caused by *S. aureus*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jinf.2015.06.005>.

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Key point

In vitro production of most phenol soluble modulin proteins is significantly higher among MRSA isolates obtained from skin and soft tissue infection when compared to MRSA isolates obtained from patients with hospital acquired pneumonia or infective endocarditis.

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Figure 1.

Spa Type Distribution Among MRSA Isolates from Skin/Soft Tissue Infection (SSTI), Infective Endocarditis (IE), and Hospital Acquired Pneumonia (HAP). 177 MRSA isolates from SSTI, IE, and HAP underwent *spa* typing, with the most common 5 *spa* types overall plotted above (n = 125; 71% of cohort). Using Chi-squared analysis, there is a significant difference in *spa* type distribution among clinical groups, with isolates from SSTI being significantly more likely to be *spa* type 1 (Ridom t008) and significantly less likely to be *spa* type 2 (Ridom t002; $p < 0.0001$).

Figure 2.

Mean Levels of *In Vitro* Phenol Soluble Modulin Production According to Infection Type of the Source MRSA Isolates. Production of PSM peptides in culture supernatant from infective endocarditis (IE), skin and soft tissue (SSTI), and hospital acquired pneumonia (HAP) isolates was quantitated and averaged over two measurements ($n = 59$ for each clinical group). Differences in average production between groups were calculated using separate Kruskal–Wallis tests for each peptide. Error bars correspond to the SEM. *Indicates lower production among SSTI isolates. **Indicates lower production among HAP isolates. Quantitation of all other peptides was highest among SSTI isolates.

Figure 3.

Mean Levels of *In Vitro* Phenol Soluble Modulin Production among USA300 Isolates vs All Other Isolates. Average production of PSM peptides in culture supernatant from USA300 MRSA isolates ($n = 44$) was compared to non-USA300 MRSA isolates ($n = 133$).

Differences in average production between groups were calculated using separate Kruskal–Wallis tests for each peptide. Error bars correspond to the SEM. *Indicates lower production among SSTI isolates. Quantitation of all other peptides was highest among SSTI isolates.

Figure 4.

Mean Levels of *In Vitro* Phenol Soluble Modulin Production According to Infection Type of the Source MRSA Isolates Excluding USA300. Analysis of average PSM peptide production was repeated for each clinical group after excluding USA300 isolates (IE n = 58; SSTI n = 24; HAP n = 51). Differences in average production between groups were calculated using separate Kruskal–Wallis tests for each peptide. Error bars correspond to the SEM. *Indicates lower production among SSTI isolates. **Indicates lower production among HAP isolates.

Table 1

Baseline characteristics of 59 patients with MRSA skin and soft tissue infection.

Parameter	Value (n)
Demographic characteristics	
Mean age (yr) $\pm$ SD	45.2 $\pm$ 17.1
Male sex	67.8% (40)
White race	83.1% (49)
Source of infection	
Deep/extensive cellulitis	13.6% (8)
Infected burn	6.8% (4)
Infected ulcer	5.1% (3)
Major abscess	54.2% (32)
Wound	20.3% (12)
Geographical location	
North America	62.7% (37)
Europe	27.1% (16)
Australia	10.2% (6)
Prior antibiotic use	
24 h of therapy	33.9% (20)
>24 h of therapy	35.6% (21)
No prior therapy	30.5% (18)
MRSA risk factors	
Hospitalization in previous 6 months	25.4% (15)
Antibiotic treatment within previous 3 months	54.2% (32)
Chronic illness	62.7% (37)
Prior MRSA infection	20.3% (12)
Admission from nursing home	1.7% (1)
Surgical procedure during current stay	17.0% (10)
Residence in area of MRSA endemicity	59.3% (35)
History of diabetes	13.6% (8)
Infection characteristics	
Fever (temp of $>38^{\circ}\text{C}$)	8.5% (5)
WBC count of $>10 \times 10^9$ cells/L	39.0% (23)
Baseline infection area $>100\text{ cm}^2$	54.2% (32)
Clinical response at point of care	
Cure	89.8% (53)
Not cured	10.2% (6)

Table 2

Baseline characteristics of 59 patients with MRSA hospital-acquired pneumonia.

Parameter	Value (n)
Demographic characteristics	
Mean age (yr) SD	65.4 ± 19.3
Male sex	61.0% (36)
Geographical location	
North America	62.7% (37)
Europe	27.1% (16)
Australia	10.2% (6)
MRSA risk factors	
Hospitalization in previous 6 months	61.0% (36)
Antibiotic treatment within previous 3 months	50.9% (30)
Chronic illness	84.8% (50)
Prior MRSA infection	13.6% (8)
Admission from nursing home	35.6% (21)
Surgical procedure during current stay	18.6% (11)
Residence in area of MRSA endemicity	35.6% (21)
Smoking status	
Current smoker	22.0% (13)
Ex-smoker	40.7% (24)
Nonsmoker	35.6% (21)
Not recorded	1.7% (1)
Mean body mass index ± SD (kg/m ²)	
On hemodialysis	3.4% (2)
In acute renal failure	6.8% (4)
Immunocompromised/severe organ dysfunction	6.8% (4)
Cardiac comorbidities	67.8% (40)
Operative status	
Nonoperative	81.4% (48)
Emergency post-operative	17.0% (10)
Elective post-operative	1.7% (1)
On ventilator support at randomization	32.2% (19)
In the ICU at baseline	49.2% (29)
Mean total APACHE II score ± SD	15.8 ± 6.0
Baseline <i>S.aureus</i> bacteremia	15.3% (9)
Outcomes at 28 days	
Alive or censored	71.2% (42)
Deceased	28.9% (17)

Table 3

Baseline characteristics of 59 patients with MRSA infective endocarditis.

Parameter	Value (n)
Demographic characteristics	
Mean age (yr) $\pm$ SD	63.2 $\pm$ 15.6
Male sex	52.5% (31)
Geographical location	
North America	62.7% (37)
Europe	27.1% (16)
Australia	10.2% (6)
Predisposing conditions	
Diabetes mellitus	44.8% (32) ^a
Hemodialysis dependent	25.4% (15)
History of cancer	15.3% (9)
Presumed place of acquisition	
Nosocomial	44.1% (26)
Non-nosocomial healthcare-associated	39.0% (23)
Community acquired	17.0% (10)
Type of endocarditis	
Native valve	61.4% (35)
Prosthetic valve	24.6% (14)
Other	14.0% (8)
Not recorded	3.4% (2)
Clinical findings	
Fever (temp of $>38^{\circ}\text{C}$)	92.7% (51) ^c
Conjunctival hemorrhage	10.3% (6) ^a
Janeway lesions	3.5% (2) ^a
Osler's nodes	3.5% (2) ^a
Roth spots	1.7% (1) ^a
Rheumatoid factor elevation	1.8% (1) ^b
Echocardiographic findings	
New regurgitation	44.1% (26)
Intracardiac vegetation	88.1% (52)
Perivalvular abscess	5.1% (3)
Complications	
Stroke	20.3% (12)
Congestive heart failure	27.1% (16)
Intracardiac abscess	10.1% (6)
Persistent bacteremia	47.5% (28)
In-hospital death	35.6% (21)

^aData not recorded for one patient.

^bData not recorded for three patients.

^cData not recorded for four patients.

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