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Increased *in vitro* phenol-soluble modulin production is associated with soft tissue infection source in clinical isolates of methicillin-susceptible *Staphylococcus aureus*

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KEYWORDS

Summary *Background:* Phenol-soluble modulins (PSM) are amphipathic proteins produced by

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Phenol-soluble modulin;
Methicillin-susceptible *Staphylococcus aureus*;
Skin and soft tissue infection;
Pneumonia;
Endocarditis

Staphylococcus aureus that promote virulence, inflammatory response, and biofilm formation. We previously showed that MRSA isolates from soft tissue infection (SSTI) produced significantly higher levels of PSM than MRSA isolates from hospital-acquired pneumonia (HAP) or infective endocarditis (IE). In this investigation, we sought to validate this finding in methicillin-susceptible *S. aureus* (MSSA) isolates.

Methods: MSSA isolates (n = 162) from patients with SSTI, HAP, and IE were matched 1:1:1 based on geographic origin of the infection to form 54 triplets (North America n = 27, Europe n = 25, Australia n = 2). All isolates underwent spa typing and were classified using eGenomics. In vitro PSM production was quantified by high-performance liquid chromatography/mass spectrometry. Fischer's Exact Test and the Kruskal–Wallis test were used for statistical analysis.

Results: Spa1 was more common in SSTI (14.81% SSTI, 3.70% HAP, 1.85% IE) ($p < 0.03$). Spa2 was more common in HAP (0% SSTI, 12.96% HAP, 3.70% IE) ($p < 0.01$). Levels of PSMα1-4 all differed significantly among the three clinical groups, with SSTI isolates producing the highest levels and IE producing the lowest levels of PSMα1-4. Spa1 isolates produced significantly more delta-toxin ($p < 0.03$) than non-Spa1 isolates. No associations between PSM levels and clinical outcome of SSTI, HAP, or IE were identified.

Conclusion: Production of PSMα1-4 is highest in SSTI MSSA isolates, supporting the hypothesis that these peptides are important for SSTI pathogenesis. These findings are similar to those described in MRSA, and demonstrate that associations between PSM levels and type of infection are independent of the methicillin-resistance status of the isolate.

Key point: *In vitro* production of most phenol-soluble modulin proteins is significantly higher among methicillin-susceptible *Staphylococcus aureus* isolates obtained from skin and soft tissue infection when compared to *S. aureus* isolates obtained from patients with hospital-acquired pneumonia or infective endocarditis.

Introduction

Staphylococcus aureus produces many virulence factors that affect its clinical manifestations.[1,2] One recently discovered family of virulence factors are phenol-soluble modulins (PSM). Members of this group of amphipathic, alpha-helical peptides may influence inflammation induction, biofilm production, erythrocyte lysis, neutrophil lysis after *S. aureus* has been phagocytosed, and even antimicrobial activity.[3] *S. aureus* is currently known to produce eight PSMs. α-type PSMs are 20–25 amino-acids long and are subdivided into PSMα1-4, delta-toxin, and PSM-mec. β-type PSMs are 44 amino-acids long and include PSMβ1-2.[3] PSMα1-4, PSMβ1-2, and delta-toxin are encoded on the core genome while PSM-mec is encoded on the mobile genetic element that confers methicillin-resistance to *S. aureus*.[4] All PSMα family proteins are encoded on the *psmα* operon, while PSMβ1-2 are both encoded on the *psmβ* operons, facilitating co-transcription of PSMα family proteins and of PSMβ1-2.[5]

We previously showed that MRSA isolates from patients with SSTI produced significantly more PSMs *in vitro* than geographically-matched MRSA isolates from patients with either HAP or IE.[6] However, our results could have been confounded by the presence of traits that were associated with the methicillin-resistance phenotype. For example, PSM-mec is present on the SCCmec element of MRSA isolates and decreases the production of PSMα peptides by inhibiting the positive regulator of PSMα-type production.[7] MRSA and MSSA isolates have also been shown to produce different amounts of enterotoxin A, alpha toxin, and toxic shock syndrome toxin.[8,9] Thus, potential associations between increased PSM production and specific infection types needs to be confirmed in MSSA isolates.

In the present investigation, we sought to confirm any such association. Our *a priori* hypothesis was that levels of *in vitro* PSM are higher in MSSA isolates from SSTI. To test this hypothesis, we utilized a unique, globally representative collection of *S. aureus* from a variety of clinical syndromes.

Materials and methods

Definitions

SSTI was defined as the presence of at least 1 of the following conditions: major abscess requiring surgical drainage, cellulitis, infected wound or ulcer, or infected burn plus purulent drainage/collection or ≥3 of the following conditions: heat, localized warmth, fluctuance, erythema, swelling, induration, pain/tenderness to palpation, temperature ≥38 °C, WBC ≥10,000 cells/mm^3, or ≥15% bands.[10] HAP was defined as: 1) presence of new or progressive infiltrates or consolidation, with or without pleural effusion on chest radiograph or computed tomography; 2) adequate respiratory specimen for culture and Gram stain and identification of an organism consistent with a respiratory pathogen isolated from respiratory tract or blood, or >2 of the following: cough, purulent sputum, auscultation findings, dyspnea, tachypnea, or hypoxemia acquired after 48 h in an inpatient acute or chronic care facility or that developed within 7 days after being discharged. In addition, patients had >2 of the following: temperature ≥38 °C or rectal/core temperature ≤35 °C, respiratory rate ≥30 breathes per minute, pulse ≥120 beats per minute, altered mental status, mechanical ventilation, WBC >10,000 cells/mm^3, <4500 cells/mm^3, or >15% band