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Panton–Valentine leucocidin expression by *Staphylococcus aureus* exposed to common antibiotics

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Summary *Objectives:* We set out to investigate the impact of common antibiotics on Panton–Valentine Leucocidin (PVL) expression by methicillin-sensitive *Staphylococcus aureus* (MSSA). PVL expression by methicillin-resistant *S. aureus* (MRSA) is reportedly enhanced by β-lactams, but inhibited by protein-synthesis inhibitors, a fact that has influenced management of infections associated with PVL. Although PVL is more frequently associated with MSSA than MRSA in the UK, the effect of antibiotics on PVL expression by MSSA has not been fully addressed.
Methods: MSSA was cultured *in vitro* with varying concentrations of flucloxacillin, clindamycin or linezolid and PVL expression measured by qRT-PCR and Western blotting. A murine MSSA abscess model was developed to measure leucocidin expression *in vivo* following antibiotic treatment.
Results: 9% (27/314) of MSSA isolates from patients with uncomplicated community skin/soft tissue infections were positive for PVL genes (*lukFS-PV*). PVL expression by MSSA in broth was unaffected by varying concentrations of flucloxacillin, clindamycin or linezolid. In a murine abscess model, treatment with flucloxacillin did, however, enhance *in vivo* MSSA *lukF-PV* transcription and this was sustained even when flucloxacillin was combined with clindamycin, or clindamycin plus linezolid. Notwithstanding increased leucocidin transcription, functional leucotoxin activity was not enhanced. Treatment with flucloxacillin plus clindamycin significantly decreased leucotoxin activity, but the addition of a second protein synthesis inhibitor, linezolid, did not confer benefit.
Conclusions: Our results suggest flucloxacillin in combination with a single protein-synthesis inhibitor such as clindamycin would give the best treatment outcome.

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Introduction

Staphylococcus aureus is a globally important human pathogen that can cause a wide spectrum of diseases attributable to the range of virulence factors it is able to express. Factors that interfere with the host innate immune response are of critical importance to the success of the pathogen.

Panton–Valentine Leucocidin (PVL) is one of four pore forming bi-component toxins that may be expressed by *S. aureus* strains. The other three are gamma-haemolysin (HlgABC), LukFS (also known as LukAB or LukGH) and LukDE. The two co-transcribed components of PVL, LukS-PV and LukF-PV, when combined can lyse human cells expressing C5a receptors, including neutrophils.[1] Strains carrying PVL typically cause suppurative skin and soft tissue infections and severe necrotising pneumonia.[2] In north America and spreading globally, PVL has been mainly associated with strains of community acquired methicillin resistant *S. aureus* (CA-MRSA)[3,4] but UK based studies suggest a more common association with community acquired methicillin sensitive *S. aureus* (MSSA) strains.[5]

While there is a broad literature that addresses production of PVL by CA-MRSA, reports investigating production by clinical MSSA strains are limited. Previous studies have shown that β-lactam antibiotics at sub-inhibitory concentrations can enhance MRSA transcription and expression of PVL and other toxins *in vitro*,[6–9] although the clinical relevance of these studies to MSSA is complicated by the fact that β-lactams have no antimicrobial activity against MRSA. Antimicrobial agents targeting protein synthesis, however, have been shown to effectively reduce transcription and/or expression of PVL *in vitro*.[6,10,11] Guidelines for treating suspected PVL MRSA infections have been influenced by these *in vitro* studies.[12] Due to the potential effect of β-lactams on toxin expression, caution is advised with regard to use in cases of PVL-associated *S. aureus* infection and the adjuvant use of one or more protein synthesis inhibitors has been recommended.[12] Although β-lactams are still the treatment of choice for MSSA, the effect of β-lactams and protein synthesis inhibitors on the expression of toxins in clinical MSSA has not yet been fully explored.

In this work we aimed to explore the effect of the commonly used β-lactam flucloxacillin and two protein synthesis inhibitors, clindamycin and linezolid, on MSSA expression of PVL and other leucocidins. *In vitro* exposure to each antibiotic at varying concentrations, including sub-inhibitory, did not yield a significant change in either transcription of *lukF-PV* or LukF-PV protein expression. However, *in vitro* exposure to antibiotics does not adequately reflect clinical exposure to antibiotics during infection. To this end we developed a murine abscess model to measure toxin transcription *in vivo*. Although PVL has no effect on murine neutrophils and cannot be used to model disease outcomes related to PVL, it can be used to measure *in vivo* expression of toxins and other *S. aureus* factors. In contrast to *in vitro* findings, we detected a higher level of *lukF-PV* transcript in mice treated with flucloxacillin compared to no antibiotic treatment. Surprisingly, addition of clindamycin or clindamycin plus linezolid enhanced *lukF-PV* transcript to an even greater level. Overall leucotoxin activity present in the abscess following antibiotic treatment was not affected by increased leucotoxin transcript and was in fact significantly decreased when flucloxacillin was combined with clindamycin.

Materials and methods

Bacterial strains

MSSA isolates (n = 314), including strains HSS03 and HSS156 used throughout this study, represented isolates from uncomplicated community SSTI collected over a one year period (2009–2010) by a single diagnostic laboratory at Hammersmith Hospital NHS Trust, London, UK (now ICHT). MSSA strains were cultured on Columbia blood agar (Oxoid) or in CCY media at 37 °C shaking at 200 rpm. Minimal inhibitory concentrations (MICs) for one MSSA PVL-positive strain collected (HSS156) were determined, in culture using a standard microdilution method,[13] to be 0.125 mg/L for flucloxacillin, 0.25 mg/L for clindamycin and 2 mg/L for linezolid.

DNA extraction

Bacteria were pelleted from overnight culture and resuspended in 100 μl of lysis buffer (100 mM NaCl, 10 mM Tris–HCl pH8, 1 mM EDTA, 1% Triton-X-100) with 2 μl of 1 mg/ml lysostaphin and incubated for 37 °C for 15 min before boiling for 10 min. Samples were centrifuged 13,000 × *g* for 2 min and the DNA-containing supernatant was further purified using an equal volume of chloroform. DNA was precipitated with isopropanol and resuspended in ddH_2O. PCR using Luk-PV primers (Table 1) was performed to test for the presence of the *lukFS-PV* genes. Separate PCR reactions were also performed using positive control housekeeping 16s primers (Table 1) to control for DNA quality.

In vitro exposure to antibiotics

Overnight cultures of *S. aureus* were diluted 1 in 10 then cultured for 24 h in CCY media. Where antibiotics were used, bacteria were cultured for 3 h before ¼ MIC, ½ MIC, MIC or 5x MIC of required antibiotic was added. Every hour samples were taken, centrifuged at 2000 × *g* for 10 min and culture supernatant 0.2 μM filtered. RNA was extracted from bacterial cell pellets using a hot-phenol method as previously described.[14] Filtered supernatant was concentrated 50 fold using Amicon ultra 10 kDa MWCO spin columns (Milipore) for Western blotting.

Recombinant LukF-PV protein

LukF-PV was amplified using LukF-recombinant primers (Table 1) and cloned into pQE-30 UA vector (Qiagen). Recombinant protein expression was induced according to the manufacturer's instructions and recombinant