

Production of phenol soluble modulins (PSMs) by community and hospital associated MRSA strains correlates with *in vivo* virulence in a *Galleria mellonella* (caterpillar) model

Eve Boakes,¹ Helene Marbach,¹ Steven Lynham,² Malcolm Ward,² Jonathan D. Edgeworth,¹ Jonathan A. Otter¹
(jonathan.otter@kcl.ac.uk)

1. Centre for Clinical Infection and Diagnostics Research (CIDR), Department of Infectious Diseases, King's College London and Guy's and St. Thomas' NHS Foundation Trust, London, UK. 2. Centre of Excellence for Mass Spectrometry, King's College London.

1. Objectives

- To evaluate *in vitro* production of PSMs and *in vivo* virulence using a *Galleria mellonella* (caterpillar) model for UK healthcare-associated (HA) MRSA clones and local and internationally established community-associated (CA) MRSA clones.

2. Methods

- Representative isolates of CA-MRSA that carry (+) or don't carry (-) Pantone-Valentine leukocidin were selected: ST1-IV(+) (USA400), ST1-IV(-) (a local CA-MRSA clone), ST8-IV(+) (USA300), ST22-IV(+), ST30-IV(+) (SWP), ST59-IV(+) and ST80-IV(+) (European clone).
- Two healthcare-associated clones were also included: ST22-IV(-) (EMRSA-15) and ST36-IV(-) (EMRSA-16).

- For the *Galleria mellonella* model, 10 μ L of overnight culture containing approximately 10^{4.5} bacteria were inoculated into groups of 12 caterpillars per isolate.¹
- Caterpillars were scored dead or alive at 16, 40, 64 and 96 hours.
- Survival times were compared using Kaplan-Meier analysis with a log rank test to assess statistical significance.
- Electrospray liquid chromatography and a multiple reaction monitoring method of mass spectrometry was used to measure relative production of PSM 1-4, 1-2 and haemolysis.²
- Mean PSM production was plotted against mean caterpillar survival time to examine the possibility of a correlation.
- Pearson's correlation test was performed to evaluate the significance of the correlation.

Table. Mean and median survival times, and matrix of p values generated from Kaplan-Meier plots comparing survival times between strains.

	Mean survival time / hours	Std. error	Median survival time / hours	P values generated from Kaplan-Meier plots comparing survival times								
				ST22 PVL+	ST22 (E15) PVL -	ST1 (t127) PVL-	ST8 USA300 PVL+	ST59 PVL+	ST36 (E16) PVL -	ST30 PVL+	ST80 PVL+	ST1 USA400 PVL+
ST22 PVL+	32.8	3.6	16	-	0.004	0.018	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST22 (E15) PVL -	41.9	1.7	40	-	-	0.754	0.005	0.021	<0.001	<0.001	<0.001	<0.001
ST1 (t127) PVL-	43.8	3.8	40	-	-	-	0.050	0.081	<0.001	<0.001	<0.001	<0.001
ST8 USA300 PVL+	56.2	4.7	40	-	-	-	-	0.516	0.203	0.153	0.014	<0.001
ST59 PVL+	57.0	4.2	40	-	-	-	-	-	0.03	0.026	0.001	<0.001
ST36 (E16) PVL -	61.9	2.0	64	-	-	-	-	-	-	0.736	0.074	<0.001
ST30 PVL+	66.1	3.9	64	-	-	-	-	-	-	-	0.222	<0.001
ST80 PVL+	73.0	4.1	96	-	-	-	-	-	-	-	-	0.001
ST1 USA400 PVL+	88.2	3.4	96	-	-	-	-	-	-	-	-	-

Figure 1. PSM production.

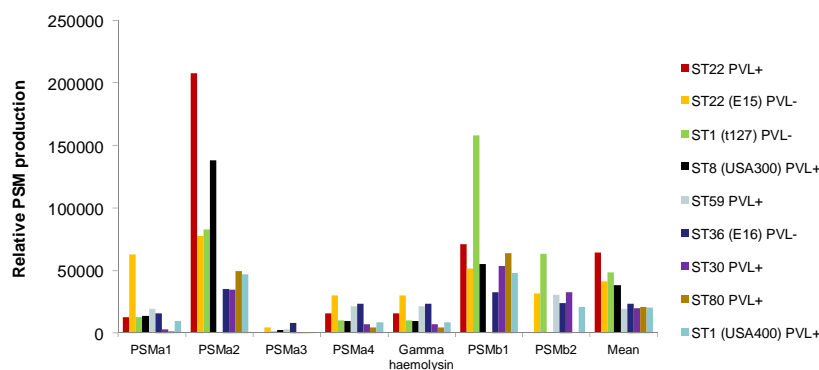
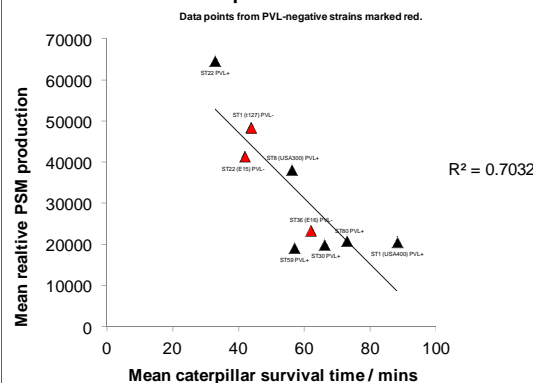


Figure 2. Mean PSM production vs. mean caterpillar survival time.



3. Results

- Mean caterpillar survival time ranged from 32.8 hours for ST22-IV(+) to 88.2 hours for ST1-IV(+) (USA400) (Table).
- PSMs were identified in supernatants of all community and healthcare strains, although PSM 2, PSM 3, PSM 1 and PSM 2 were absent from one or more strains (Figure 1).
- ST22-IV(+) was significantly more effective and ST1-IV(+) (USA400) significantly less effective at killing caterpillars than any other strain.
- Surprisingly, ST22-IV(-) (EMRSA15), the most common healthcare-associated MRSA clone in the UK, had the 3rd highest PSM production and was the second most effective caterpillar killer.
- There was a significant negative correlation between mean PSM production and mean caterpillar survival time ($r^2=0.70$, Pearson's Correlation $p=0.01$) (Figure 2).

References

- Peleg *et al.* *J Infect Dis* 2009;199:532-536.
- Wang *et al.* *Nat Med* 2007;13:1510-1514.
- Labandeira-Rey *et al.* *Science* 2007;315:1130-1133.
- Diep *et al.* *PNAS* 2010;107:5587-5592.
- Cheung *et al.* *Microbes Infect* 2012;14:380-386.
- Cheung *et al.* *Infect Immun* 2011;79:1927-1935.

4. Conclusions

- The bacterial factors underlying virulence in *S. aureus* are not well understood.
- Several studies indicate that PVL plays a key role in virulence.³⁻⁴
- The ability to kill caterpillars was not obviously linked to PVL carriage; however, there was a statistically significant negative correlation between overall production of PSMs and caterpillar survival time.
- Other studies have indicated that PSMs may play a key role in the virulence of *S. aureus* infections.^{2,5}
- The *G. mellonella* virulence model is potentially a useful high throughput model to assess *S. aureus* virulence.
- We plan to evaluate whether the expression of RNAlII from *agr* correlates with our findings.⁶
- Further work is required to correlate virulence in *G. mellonella* with human disease phenotypes.