P2169

Abstract (poster session)

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

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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. Methods: Representative isolates of CA-MRSA that carry (+) or don't carry (-) Panton-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, 10µL of overnight culture containing approximately 10^4.5 bacteria were inoculated into groups of 12 caterpillars per isolate. 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 alpha1-4, beta 1-2 and gamma haemolysis. Results: PSMs were identified in supernatants of all community and healthcare strains, although gamma haemolysis was not detected in 6/9 strains and PSM beta2 was not detected in 3/9 strains. Mean caterpillar survival time ranged from 32.8 hours for ST22-IV(+) to 88.2 hours for ST1-IV(+) (USA400) (Table). 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 (r2=0.63, Pearson's Correlation p=0.01). Conclusion: The G. mellonella virulence model is potentially a useful high throughput model to assess S. aureus virulence. 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. Further work is required to correlate virulence in G. mellonella with human disease phenotypes.

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

				P values generated from Kaplan-Meier plots comparing survival times								
	Mean survival time / hours	Std. error	Median survival time / hours	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	-	-	-	-	-	-	-	-	-