

Panton-Valentine Leucocidin positive CC121 strain Methicillin-sensitive *Staphylococcus aureus* is associated with Pyomyositis in Cambodian children

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INTRODUCTION

- Pyomyositis is a severe infection of skeletal muscle that affects young children in tropical regions
- Up to 90% of pyomyositis is caused by *Staphylococcus aureus* (*S. aureus*)
- Genetic tools have evolved and provide a novel opportunity to examine the relationships between entire bacterial genomes and virulence
- We designed a case control study using *S. aureus* strains causing pyomyositis in children attending the Angkor Hospital for Children (AHC), Siem Reap, Cambodia together with nasal carriage strains collected over the same time period from the same human population visiting AHC as outpatients
- Aim: identify genes associated with virulence in *S. aureus* strains causing pyomyositis in Cambodian children undertaking a bacterial Genome Wide Association Study (GWAS) approach

METHODS

Sample sets

1) Pyomyositis (case isolates)

- All cases identified retrospectively via ICD-10 discharge codes of M60 between January 2007 and December 2012 with *S. aureus* isolated from pus samples taken from a skeletal muscle abscess were considered; clinical notes were reviewed and cases consistent with pyomyositis included
- 106 patients with pyomyositis identified; 102 non-duplicate *S. aureus* isolates sequenced

2) Nasal carriage (control isolates)

- Two sets of carriage strains collected from outpatients attending AHC outpatients:

- Carriage study performed in 2008¹

Anterior nares of 2,485 children swabbed; 519 *S. aureus* strains isolated; 222 *S. aureus* isolates sequenced

- Carriage study performed in 2012

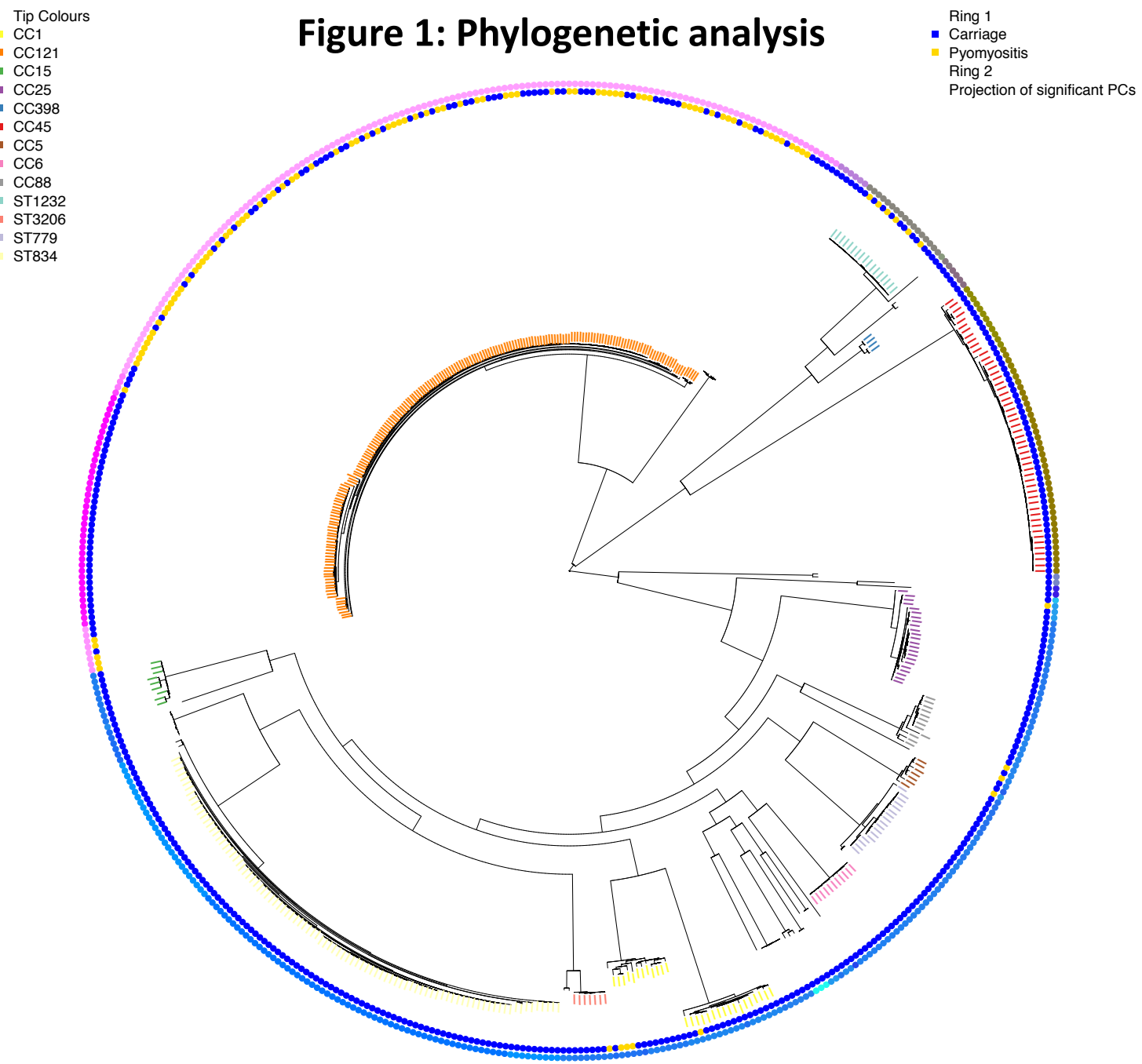
Anterior nares of 1058 children swabbed; 261 *S. aureus* strains isolated; 196 *S. aureus* isolates sequenced

Ethical approval was granted by the AHC Institutional Review Board and the Oxford Tropical Research Ethics Committee

Microbiology and Genomics Methods

- Antimicrobial sensitivity testing performed following CLSI guidelines
- Single colonies were picked, DNA extracted² and randomly allotted a well in the sequencing plate
- All isolates were sequenced on the Illumina HiSeq 2500; reads mapped to USA300 CA-MRSA reference genome³ and assembled using Velvet
- GWAS was performed controlling for relatedness between the strains using GEMMA⁴: testing for strain associations using *Bugwas*⁵, and for associations of SNPs and 31bp kmers⁶ (unique 31 base pair DNA sequences) to fully capture diverse genetic elements

RESULTS



- Examines every unique 31 base pair kmer in the *de novo* assembly testing for association with case/control status after controlling for population structure
- Strongest signal of association in kmers mapping to the *lukF-PV* and *lukS-PV* genes of the Panton-Valentine leucocidin (PVL) operon and the upstream region of these genes (OR=131-142 (p=10^{-17.9} - 10^{-21.5}))

DISCUSSION

Using bacterial GWAS we have uncovered strong evidence of strain-to-strain variability in *S. aureus* causing pyomyositis: the susceptible CC121 strain most commonly causes pyomyositis in Cambodian children. We show a major association between the presence of PVL and its upstream region in *S. aureus* strains isolated from Cambodian children with pyomyositis.

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