EP0045
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Invasive Staphylococcus aureus disease is associated with genomic changes arising within hosts

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Background: Invasive *Staphylococcus aureus* disease is a cause of significant morbidity and mortality, but is nevertheless uncommon relative to *S. aureus* carriage, which has an incidence of 30%. We previously reported regulatory gene changes in a case of transition from *S. aureus* carriage to bacteraemia. We hypothesised that these may be functionally important in pathogenesis, and within-host evolution, particularly in regulatory pathways, may increase the virulence of *S. aureus*.

Material/methods: 105 individuals with *S. aureus* cultured from both nasal swab and clinical samples were identified from the clinical laboratory of two UK hospitals. Clinical samples were blood culture (n=55) and pus, soft tissue, bone or joint samples (n=50). Five or more individual colonies from each culture underwent whole genome sequencing on the Illumina HiSeq platform, yielding 1143 sequences. Variants were detected both by mapping reads to reference strains and *de novo* assembly. Maximum likelihood trees were constructed for each individual. Variants were typed for position on the reference genome, predicted effect on protein transcription (silent, non-synonymous and protein truncating – including both nonsense mutations and frameshift indels resulting in premature stop codons). Variants were also typed for location on the tree (within carriage, within disease, between carriage and disease). We tested each branch type for an excess of variants in: individual loci; loci with shared gene ontology; loci within shared transcriptional response (identified by microarray studies).

Results: 95 individuals had carriage and invasive populations differing by 66 or fewer variants, while the remaining 10 individuals showed unrelated carriage and disease populations. We identified 1324 variants: 615 within carriage, 171 within disease and 538 between related carriage and disease populations. Branches between carriage and disease showed enrichment for truncating variants in accessory gene regulator protein A (*agrA,* p=9.8x10^-8), non-synonymous variants in cell wall genes (p=6.73x10^6) and fifteen transcriptional pathways. Seven of these pathways are under the control of transcriptional regulators (see table), including Staphylococcal accessory regulator R (SarR), *agrA* and

Repressor of surface proteins (Rsp). In addition, we found an excess of variants among eight groups of genes with altered transcription in environmental stress or antibiotic exposure. In contrast, branches within carriage and within disease were not enriched for variants in any transcriptional regulators or regulatory pathways.

Conclusions: Subtle changes in the transcriptional regulation of *Staphylococcus aureus* arise within hosts and are associated with the development of invasive disease.

Variants	Transcriptional response of genes showing variation between carriage and dsiease	P-value
Non-synonymous	Down-regulated in rsp knockout	4.59x10^-6
Truncating	Down-regulated in agrA knockout (UAMS-1)	5.73x10^-6
Truncating	Down-regulated in arlR knockout	7.04x10^-6
Non-synonymous	Down-regulated in agrA knockout (RN27)	4.05x10^-5
Truncating	Down-regulated in airR mutant	4.55x10^-5
Non-synonymous	Down-regulated in SarR knockout	7.79x10^-5
Truncating	Up-regulated in mgrA knockout	1.51x10^-4