Comparative Genomic Analyses of Keratitis-Associated *Staphylococcus aureus* Isolates

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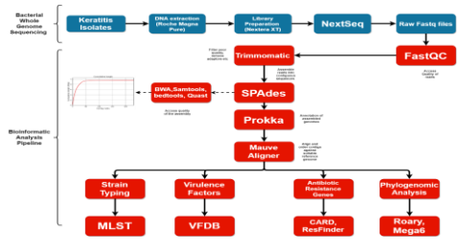
Introduction

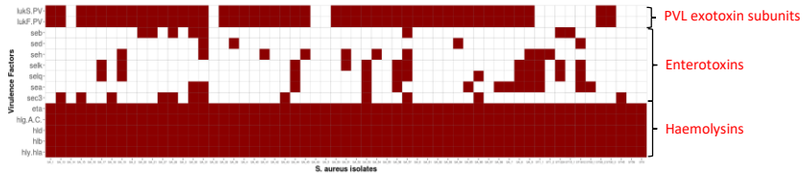
Microbial keratitis is a major cause of corneal opacity and blindness worldwide. It is capable of extremely rapid progression and so treatment must be commenced as swiftly as possible. There are a variety of causative microorganisms, with bacterial infections tending to predominate mainly due to the misuse of contact-lenses. Among the most common infective agents are opportunistic pathogens such as *Staphylococcus aureus* which infiltrate the corneal stroma, leading to an inflammatory response. Whole genome sequencing (WGS) can provide important insights into the pathogenesis of the disease. Analysis of the entire genome of an organism can confirm the presence/absence of specific virulence or antibiotic resistant determinants. Due to the high prevalence and associated morbidity of *S. aureus* ocular infections, a wider understanding of the common genetic determinants and their specific evolutionary lineages is essential in order to improve disease surveillance and provide opportunities for the development of novel therapies. Here we carry out genomic analyses on a cohort of 60 *S. aureus* samples, isolated from clinical corneal infections.

Methods



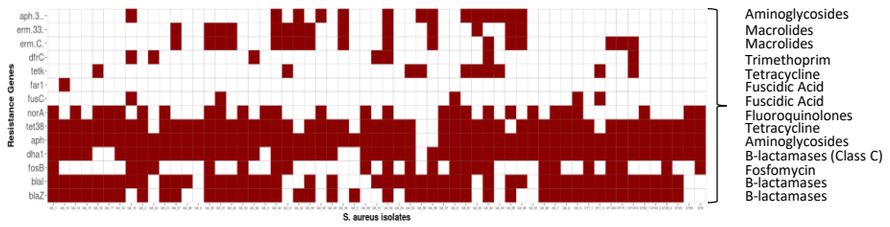
Whole genome sequencing data was obtained for 60 *Staphylococcus aureus* keratitis isolates using a miniaturized version of the Nextera XT protocol. In summary, our protocol utilized Echo acoustic liquid handling technology (Labcyte) to transfer low-volume reagents, facilitating input DNA quantities of only 50 pg. Sequencing was performed on the NextSeq platform and output fastq files were filtered for high quality reads, assembled into contiguous sequences using SPAdes and annotated using Prokka. Numerous databases were used to identify key genes involved in virulence of the disease or resistance to antimicrobial treatment. Pangenome analysis was completed using Roary software with Mega6 used to construct phylogenetic trees.

Virulence Gene Profile



A common core group of haemolysins is evident across all our *S. aureus* isolates. This includes the likes of the key pore forming α-toxin *(hla)* present in virtually all infective strains and a major known contributor to the invasion of the pathogen into the corneal stroma. The presence of superanitgen-encoding genes, as expected, is much more variable due to their tendency to be carried on various mobile genetic elements (MGEs). Also identified was the presence of the Panton-Valentine Leukocidin (PVL) exotoxin which is associated with more severe corneal infections.

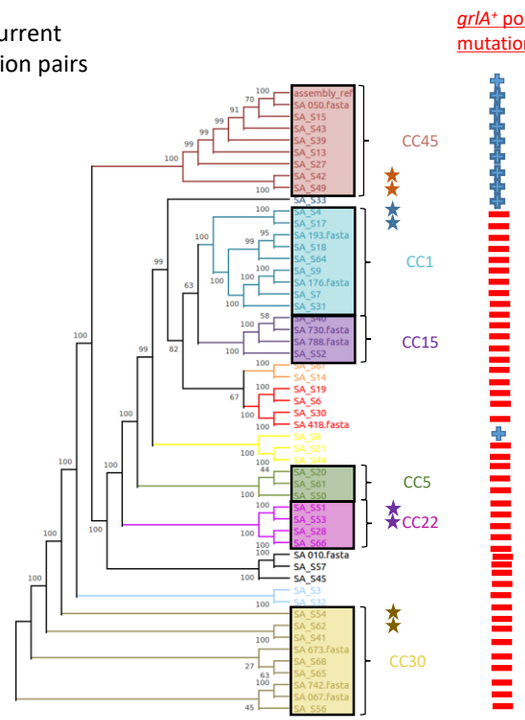
Antibiotic Resistance Determinant Profile



Multidrug resistance, defined as resistance to three or more classes of antibiotics, was documented for over 90% of this cohort of isolates. High rates of genotypic resistance to penicillin *(blaZ)* and fosfomycin *(fosB)* were observed. All strains were determined to be methicillin susceptible (MSSA) based on the lack of the *mecA* gene commonly carried on SCC cassette elements.

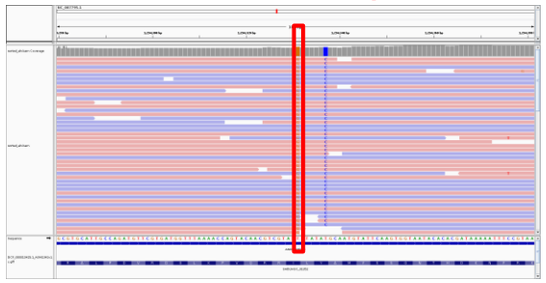
Phylogenetic Analysis

★ = Recurrent infection pairs



Sequence types were determined using a multilocus sequence typing (MLST) approach and a high resolution phylogenetic tree was constructed by performing a multiple alignment of the core genome SNPs from this cohort of *S. aureus* isolates. These isolates are grouped across six different major complex lineages – clonal complex 45 (CC45), CC1, CC15, CC5, CC22 and CC30. These include the genotypes most frequently associated with invasive disease and postsurgical infections. Isolate pairs from recurrent infection are also indicated, in all cases these were found to be closely related, originating from the same complex with identical sequence types. Recurrence may potentially be a result of endogenous site colonisation from sources such as the conjunctiva or nasal pathways. The isolates which harboured the *grlA* point mutation are also indicated, heavily concentrated towards the CC45 complex, suggesting a more resistant evolutionary subgroup.

Consideration of Key Chromosomal Mutations



The *grlA* gene encodes for the production of DNA topoisomerase IV subunit A which is involved in the regulation of topological links between DNA strains. This is the main target of the commonly used antibiotic flouroquinoline ciprofloxacin. A point mutation at the shown location is associated with increased resistance to ciprofloxacin and this was identified in 17% of this *S. aureus* cohort.

Nucleotide change: ATT → ATG

Amino Acid change: R I L → R M L

Conclusions/Future Work

- WGS provides an extremely high level of detailed information at the genome level of an organism and thus has great clinical potential. Here we have highlighted several applications based around comparative analysis and the identification of key genes which play a role in pathogenesis.
- The genetic characterisation of a diverse set of *S. aureus* ocular pathogens provides important information into the distribution of virulence and antibiotic resistance determinants, along with the common lineages associated with keratitis.
- Performing metagenomics directly upon clinical keratitis specimens offers another exciting prospect which would overcome many of the current diagnosis issues. Preliminary work is ongoing to determine the feasibility of this in terms of recovering sufficient microbial DNA and the depletion of host cells from clinical samples.