Staphylocoagulase positivity is associated with staphylocoagulase genotype in *Staphylococcus aureus* : A pilot study.

Carly Botheras[1,2]*, Dieter Bulach[3], Eugene Athan[1,2,4]

[1]Centre for Innovation in Infectious Disease and Immunology Research, [2]Deakin University, School of Medicine, IMPACT, Institute for Innovation in Physical and Mental Health and Clinical Translation, [3]Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology, The University of Melbourne [4]Public Health Directorate, Barwon Health

* carly.botheras@deakin.edu.au

Introduction

Staphylococcus aureus (S. aureus) bloodstream infections have a 30-day mortality rate of 13.5% (1), highlighting the clinical importance of the bacteria.

Since the 1950's, *S. aureus* has been differentiated from the less clinically relevant coagulase negative staphylococcus species through the detection of coagulation production (2).

Surprisingly, *S. aureus* strains falsely test negative in this coagulase tube production test (3,4).

The key significant coagulating factor that is tested in this test is staphylocoagulase, a conserved protein that is composed of conserved C and N terminals, a tandem repeat region and a variable D1, D2 and central region (4,5).

This varied region can be used to genetically categorise *S. aureus* (genotyping) (4,5).

In this study we hypothesised that the variable regions of the staphylocoagulase gene are associated with negative coagulase test results

Methods

Clinical strains of *S. aureus* were collected as part of a prior study.

To identify coagulase positivity, 126 isolates were tested for coagulase production as per manufacturers instructions at 4 and 24 hours (2).

All isolates were tested in biological triplicate.

To identify the staphylocoagulase genotype, DNA was extracted from all isolates and the whole genomes were sequenced via an Illumina MiSeq platform.

Sequencing data were placed in the Nullarbor Bioinformatic pipeline for alignment, annotation of the genome, and Multi-Locus Sequence Type (6).

Staphylocoagulase genes were extracted from the annotated data and aligned with different references genotypes, using MEGA and ClustalW bioinformatic software

To observe the closeness of the different genotypes of staphylocoagulase, phylogenetic trees were produced using FASTTree and Interactive Tree of life bioinformatic software.

Genotypes were associated with coagulase tube production positivity using univariable binary logistic regression in SPSS v29.

Graphical presentation of data was produced using GraphPad prism v8.

Results

All isolates were found to have the staphylocoagulase gene

The staphylocoagulase gene was conserved among MLST lineages

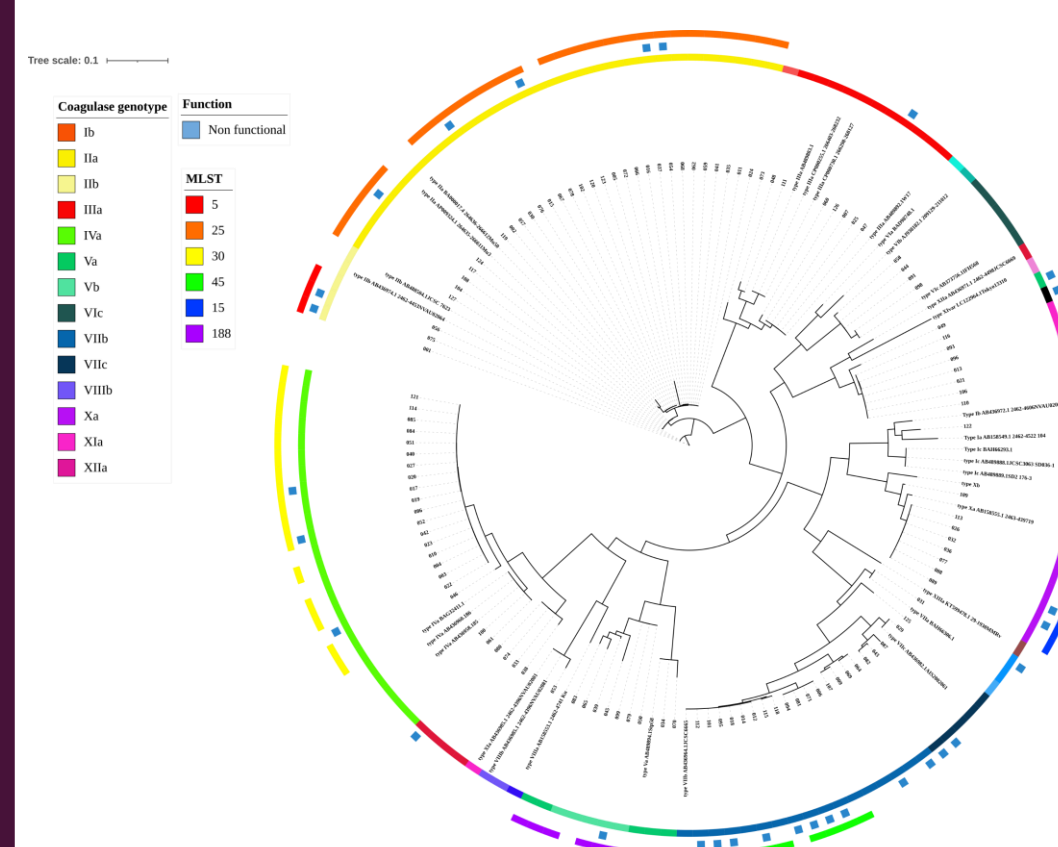31.1% of isolates tested negative via the coagulase tube test.

Figure 1. Phylogenetic Tree of different staphylocoagulase genotypes. Staphylocoagulase genes were extracted from bioinformatic data and mapped against reference genotypes. Superimposing MLST data over the tree found that genotypes were conserved across MLST types. Genotype II was the most common genotype.


Coagulase Test of different staphylocoagulase genotypes

Figure 2. Heat map of coagulase positivity percentage of different staphylocoagulase genotypes. Each Staphylocoagulase genotype was categorised by type and the positivity percentage was identified. Genotypes with more than 4 representative isolates were included in the map.

Genotype IV had a 2.96 OR (95% CI: 1.12,7.75, p=0.028) of having an early positive result.

Genotype VII had a 4.33 times OR (95% CI: 1.65, 11.35, p=0.003) and Gentoype X had a 10.93 OR (95% CI:2.19, 54.42, p=0.003) of having a negative result.
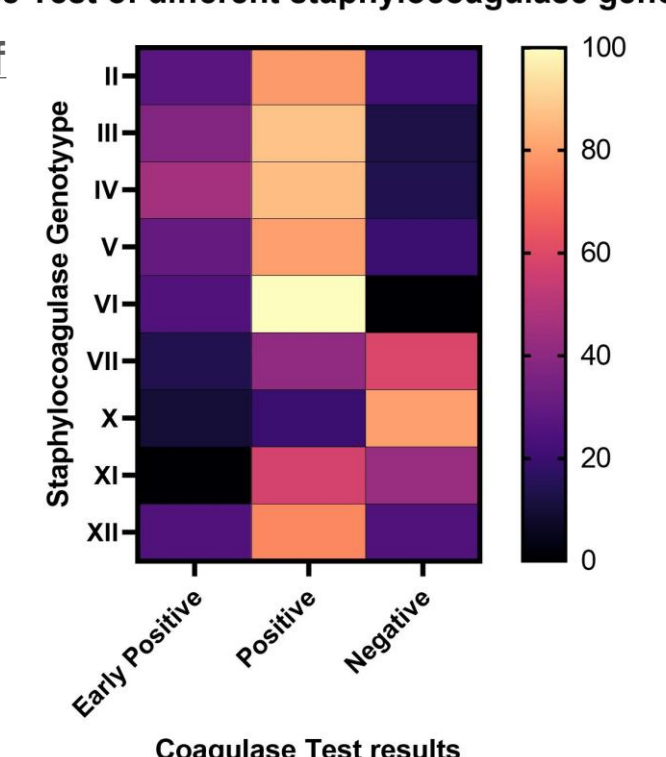
Discussion

In this small pilot study, the second most common genotype, genotype IV, was associated with early coagulase positivity and types VII, and X were associated with negative coagulase production.
The staphylocoagulase gene was co-evolved with the MLST types, suggesting that specific lineages that will be false negatives.

As the commercial coagulase test uses rabbit plasma (2), it may be that the negative types have a higher affinity for human plasma, compared to other species.
It also may be that the coagulase production in these types are more fragile and dissolve in handling, a commonly observed problem (2).

Measure expression of the staphylocoagulase gene to confirm production of coagulase as well as a larger sample size are required.

This study presents early associations that specific lineages affect positivity of the coagulase tube test.

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