S. Aureus (Staph) Genomic DNA can be recovered from Red Blood Cells

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Introduction

Background and Significance

- Staphylococcus aureus is a Gram-positive bacteria present in the nose for 30% of adults and 20% on skin.
- Spread by direct contact, a contaminated object or through droplets from sneezing or coughing.
- The most common infections are mild skin infections.
- However, they can become serious or even fatal when they lead to sepsis or osteomyelitis when bacteria spread to the bloodstream
- Antibiotics commonly prescribed to treat staph infections.
- However, staph is a rapidly adaptable bacteria and through mutation become resistant to other traditional antibiotics
- Like MRSA because they cannot be treated with antibiotics and can lead to fatal infection such as sepsis.
- Current diagnostics for severe infections are using blood samples and culturing the bacteria to identify its strain
- Further understanding of the mechanisms of staph infection and their relationship with red blood cells could lead improvements in diagnostics with reduced wait time and better accuracy

Methodology

Cell Counting, Bacterial DNA binding, DNA extraction and qPCR

1. Cell counting
- Unexpired leukoreduced blood was diluted with 90ul of PBS and counted under a microscope - Samples were centrifuged at 10k rmp for 5 min and the pellet was saved

2. Bacterial DNA binding on Human RBCs
- Sample pellets were resuspended in lo-bind tubes 100ul of PBS/tube.
- Experiment 1 used dilutions 10 ng and 1ng and incubation times of T-30, T-1, T-2, T-3, T-4.
- Samples were incubated at 37C on a rocking nutator
- Samples were overlayed on 500ml of 30% sucrose cushion and centrifuged at 13k rpm for 3 min at 4C.
- 200ml of SN was harvested from the top and RBC pellet was saved and stored.

3.DNA extraction
- For RBC pellet: pellet was resuspended
- 20ul of proteinase K and 200ul of AL added and incubated for 1hr at 56C
- DNA was extracted using Qiagen DNeasy kit and the eluate was saved

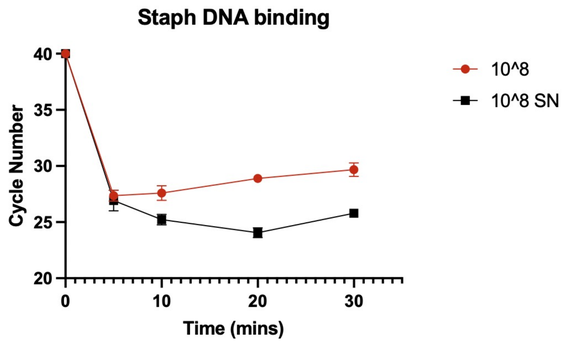
4. qPCR for bacterial binding
- primers and probes (Fig 1) were diluted to 1:10 with water and master mix was created using TaqMan
- 5.9ul of master mix and 4.1ul of each samples was pipetted into wells. Samples were ran in triplicates.
- 4 standards, H2O and RBC were included as controls
- qPCR was run for 1hr

Bacteria	FORWARD set 1:	REVERSE set 1:	PROBE set:
S. aureus (Staph) (16sF1/16sR)	TCGGMTCGTAAAACTCTGTT	CTGCTGGCACGAAGTTAGC	/56-FAM/AAGAACATA/ZEN/TGTGTAAGTAACTGTGCACA/31ABkFQ/

Fig 1. Staph primers and probes

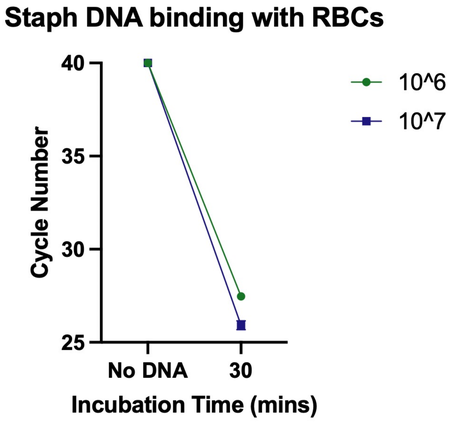
Results

Experiment 1: Staph DNA binding at incubation times of 5, 10, 20 and 30 minutes

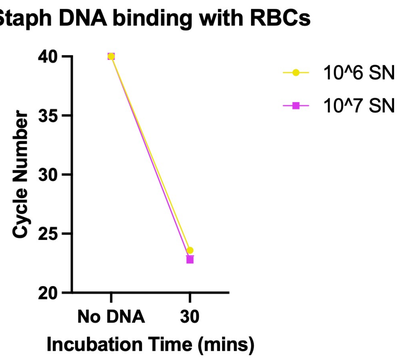


The first experiment was used to determine whether binding of staph DNA occurs and if so at which incubation times. The results demonstrate binding occurs extremely early as it can be registered at only 5 minutes which increases by 2.5 cycle numbers after another 25 minutes. This confirmed DNA does bind to red blood cells almost immediately.

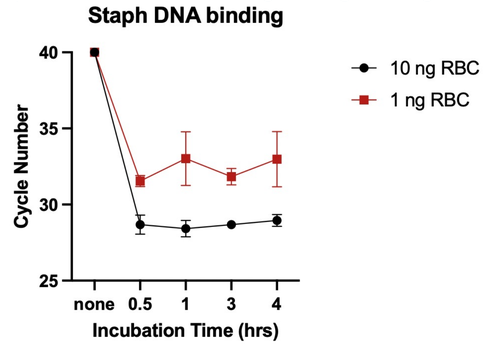
Experiment 2: Staph binding at RBC concentrations of 10^6 and 10^7



The second experiment was used to understand if there was a dose dependent acquisition relationship between the DNA and red blood cell concentration. Two different concentrations of red blood cells from the same human blood sample were compared. The results show both 10^7 RBC and SN has the lowest cycle number at 30 minutes. This suggests that there is a direct correlation between RBC concentration and binding, where the higher the concentration the more effective the binding of staph DNA to blood.
Furthermore results show that the genomic DNA has binded however it has not been sequestered. This can be seen because the cycle number of SN is not directly proportional to that measured from the RBC. Following this experiment, it was concluded higher concentrations of RBC are more effective for binding



Experiment 3: Staph Binding at 10ng and 1ng



Once it was determined that binding was dose dependent on RBC concentration, the final experiment was used to find if it was also dose dependent of DNA. Therefore two different concentrations of staph DNA, 10 ng and 1ng. From the data, 10ng is more successful at binding because it had a lower cycle number for each incubation time. This demonstrated binding is also dose dependent of DNA and higher concentrations are more successful at binding.

Discussion

MRSA remains a worldwide health issue and there has been a significant effort to improve diagnostic assays and to develop new antimicrobial agents for treatment of disease. Diagnosis for infections other than skin require blood samples to grow the bacteria and test it for staph specific structures for identification which takes 24-48 hours. It is important to determine the type of staph bacteria causing the infection in order to prescribe the correct antibiotics and prevent further antibiotic resistant strains

These experiments demonstrated staph genomic DNA binds very early on to red blood cells. If further research can be done to determine how staph DNA can be recovered from red blood cells, then detection of staph infections from blood samples could provide a quicker and perhaps more accurate diagnosis of staph infections.

Acknowledgements and References

Malachowa N, DeLeo FR. Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci. 2010 Sep;67(18):3057-71. doi: 10.1007/s00018-010-0389-4. Epub 2010 Jul 29. PMID: 20668911; PMCID: PMC2929429.

CDC https://www.cdc.gov/hai/organisms/staph.html