The Public Health Significance of the Fibrinogen-binding MSCRAMMs of *Staphylococcus aureus*

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ABSTRACT

Infective endocarditis (IE) is a bacterial infection that has existed for multiple centuries and is a disease that has been constantly evolving. Recently, cases of IE caused by *Staphylococcus aureus* have been increasing gradually. To add to the problem, little is known about the mechanism in which *S. aureus* causes the heart infection. This study examined four different receptor proteins within the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family (ClfA, ClfB, FnbpA, FnbpB), that are present on the cell wall of *S. aureus* to understand each of their role when binding to fibrinogen (Fg), a key ligand in human hosts. The differences in binding to Fg for 7 different clinical isolates of *S. aureus* were analyzed using a 96-well microtiter plate apparatus. The binding to Fg for 5 reference strains of *S. aureus* (Newman strains and 8325-4) were also examined in the study. It was determined that the Newman strain that lacked ClfA had lower absorbance readings, indicating lower binding, than ones with ClfA and ones that lacked ClfB. In addition, clinical isolates, with an exception of one, exhibited comparably higher absorbance readings, indicating enhanced binding, relative to the Newman strains. The results pointed out that ClfA plays a critical role when binding to Fg, and both FnbpA and FnbpB are also equally important. Furthermore, it was discovered that the origin of the clinical isolates impacted binding to Fg. *S. aureus* specimens from the nasal cavity had lower absorbance readings compared to isolates that originated from the bloodstream of human patients.

INTRODUCTION

Infective endocarditis (IE), a bacterial infection of the endocardial surface, was first described in 1885 by William Osler [12]. Since then, the disease has been evolving in various ways and has become a disease that is seen more commonly throughout the world. Today, IE affects 3 to 10 per 100,000 per year and there are 40,000 to 50,000 new cases each year just within the United States [1]. IE epidemiology has changed over the last several decades where now, the patient demographic is older (mean age 57.2) and prominently men (66.3% in the early 2000s) [12]. Furthermore, the microbiology of the IE seen today has changed. *S. aureus* has become the most common causative agent where it accounts for over 30% of all cases [1]. Enterococcal IE along with coagulase-negative staphylococcal IE have also increased over the years. On the other hand, *Streptococcus viridans* IE has decreased in percentage over the span of five decades, but is still the causative agent for approximately 20% of all cases [12]. While certain changes have been observed with IE over the years, many characteristics of the disease are still uncertain. To begin with, the general method of infection development is understood to some extent, but it is unclear how exactly the bacteria cause the infection within the heart.

The development of the disease can be subdivided into three steps. The initial step is bacteremia, where the bacteria enters the bloodstream through the mouth, gastrointestinal tract, skin, or urinary tract. Although less common, venous catheters as well as surgeries are also

methods of entry into the bloodstream. In the second step, the bacteria adhere to the endocardial surface. Normal endothelial lining of the heart is resistant to the adherence of bacteria; however, damaged or abnormal endocardial surfaces allow the binding of the bacteria [1]. Host proteins such as fibrin, which is composed of polymerized fibrinogen molecules, help the bacteria to bind to host or artificial (e.g. implanted) surfaces [3]. The last step is the development of biofilms where with quorum sensing and synchronized gene expression, the bacteria successfully assemble and go through maturation. Once biofilms are developed, these bacteria are protected from the host immunity and are extremely difficult to kill with antibiotics.

IE can be classified differently according to anatomical site (left/right side), diagnosis status (definite/possible), causative microbial agent, and whether it is a native valve IE or prosthetic valve IE (PVE). Among these different classifications of IE, PVE is a public health challenge that must be addressed urgently due to its high morbidity and mortality rates potentially caused by the obstacles faced with diagnosis and treatment. With the advancements in diagnosis and treatment, the mortality rate of PVE has in fact decreased to 22.8% by the beginning of the 21st century [8]. Nevertheless, despite improvements in diagnosis and treatment methods for PVE, the optimal methods are still under debate. Currently, the diagnosis of PVE is based upon positive blood culture and detection of vegetation, paravalvular abscess, fistula, or valve dehiscence through echocardiography [8]. As for treatment, antibiotic prophylaxis and/or early surgical

treatment is recommended [1]. Promising results with these methods have been observed in numerous cases, but there are still many complications with them as well.

To develop better diagnosis and treatment methods for PVE, more research must be conducted on the microbial agents from various perspectives. One approach focuses on MSCRAMM (microbial surface components recognizing adhesive matrix molecules) of *S. aureus*. This gram positive, opportunistic pathogen is the most common microorganism that causes PVE and other IEs. MSCRAMMs are known to be the main virulence factor that allows *S. aureus* to bind to proteins within the heart such as fibrinogen (Fg). If more research on these adhesive matrix molecules are conducted and the mechanism of them become apparent, the results could be used to develop new strategies to lower PVE incidence and mortality rates. This study, therefore, focused on four MSCRAMM (FnbpA, FnbpB, ClfA, and ClfB) from *S. aureus* to understand their functions and structures that could be contributing toward the binding to Fg.

These MSCRAMMs contain a signal sequence, the ligand-binding A region composed of three domains (N1, N2, and N3), the serine-aspartate repeat region (R region), and the C-terminal that is needed for cell wall anchoring (Figure 1). The A region is responsible for the Fg binding mechanism termed “Dock, Lock, and Latch” [7]. The Fg ligand docks into the trench located between the N2 and N3 domains and the C-terminal extension of the N3 domain moves over to the the ligand peptide to “lock” it in place [4].

Multiple MSCRAMMs all contribute to the binding process to Fg. However, little is known about the mechanism that allows *S. aureus* to bind to fibrinogen within the human body. This current study was conducted to understand the effects that the four MSCRAMMs chosen for the study, which were FnbpA, FnbpB, ClfA, and ClfB, have during the Fg-interaction through experimentation results with fibrinogen. The genetic compositions of each MSCRAMM were also examined to determine whether the unique gene sequences of the MSCRAMM allow some isolates of *S. aureus* to bind stronger to fibrinogen. Furthermore, relationships between clinical isolates collected from the nose and isolates obtained from the bloodstream were also analyzed since healthcare providers are currently very interested in differences observed for bacterial isolates collected from different locations in the human body.

METHODS AND PROCEDURE

Selection of bacteria samples: Specific clinical specimens of *S. aureus* were selected for these studies. Several criteria were utilized to select these particular isolates. The first and most important criteria when deciding the isolates was the amino acid sequences at the C-terminus of the subdomain N3. The C-termini of N3 for ClfA, ClfB, FnbpA, and FnbpB all play an important role during the "lock, dock, and latch" mechanism used by *S. aureus* to bind to fibrinogen. During the binding process, the C-terminus of N3 goes through a conformational change and this allows *S. aureus* to lock the peptide ligand into the trench between N2 and N3 [5].

The amino acid sequences of the A domain for both FnbpA and FnbpB were first analyzed and the samples were reorganized into groups with the same N3 C-termini. From this analysis, it was determined that the C-termini were the same for samples within the same bacterial lineage, as estimated by their clonal complex (Figure 2). The list was then compared with the list of samples that was, at that time, available in the lab's -80°C freezer. Samples that existed on both list and belonged to different locations of collection (anterior nares or bloodstream) were chosen. When selecting the samples, the clonal complex of the samples were also noted to make sure to have samples that exist in the same clonal complex, but were also clinically different in terms of whether the sample came from the blood or the nose.

Clinical samples are important to analyze because these are the ones associated with the diseases. However, their genomes are not well characterized as type strains or reference strains. Therefore, numerous reference strains were analyzed concurrently to provide a benchmark. Four different Newman strains of *S. aureus* were selected: Newman wild, ΔClfA, ΔClfB, and ΔClfA and ClfB. The wild-type *S. aureus* (8325-4) and negative mutants for the Fnbps (ΔFnbpA and ΔFnbpB) were also examined; however, the results conveyed that the negative mutants were not as critical in binding to Fg as the clumping factor proteins (see Results section).

The *S. aureus* samples of 8325-4 (wild), 250 (CC97 CDC), 172 (CC8 CDC), 3450 (CC8 CDU), 4495 (CC8 CDI), 289 (CC5 CDC), 3854 (CC5 CDU), 204 (CC5 CDC), Newman wild,

Newman ΔClfA, Newman ΔClfB, and Newman ΔClfA and ClfB were used for the experiment. CDC stands for cardiac device control, and these isolates were obtained from the nares of healthy patients with a cardiac device. CDI and CDU were obtained from the bloodstream of patients with infected (CDI) or uninfected (CDU) cardiac implants, where "infection" was defined clinically.

Use of microtiter plate assay for adhesion: Two days prior to the experimentation date, 100µl of 100µg/ml Fg solution were poured into each well of the microtiter plate. The number of wells used per plate differed every experiment since the number of samples being examined were different each time. The microtiter plate was incubated at 4°C for 24 hours. The plate was rinsed with PBS 3x followed by 200µl of 2% BSA which were poured into the same wells. After another 24 hour incubation period at 4°C, the wells were rinsed with PBS 3x again.

S. aureus isolates and reference strains were grown in tryptic soy broth (TBS) supplemented with 0.25% dextrose, incubated at 37°C at 200 rpm for 12 hours, passed to fresh TBS, and then left in TBS with 0.25% dextrose to grow until OD_{550} of 0.540 ± 0.05. Once the required absorbance level was observed, the solutions were placed into 2 centrifuge tubes and the bacteria was isolated from TBS through centrifugation at 5000 rpm for 3 minutes in 4°C. The resulting pellets were then combined into one tube with the usage of PBS. The PBS solution was then diluted until 1.000 at OD_{600}.

An amount of 50µl of the diluted bacteria were injected into 4 wells of the microtiter plate.

Then the 96 flat-well bottomed microtiter plate was incubated at 37°C for 90 minutes. After the incubation period, each well of the plate was rinsed with 4°C PBS 3x. 100µl of 2.5% formaldehyde was poured into each well and after the aspiration of the formaldehyde, 200µl of crystal violet was poured into each well. PBS was then used to rinse the wells 6x in total. Finally, 100µl of DMSO was added to each well and the microtiter plate was analyzed through Gen5™ Microplate Reader and Imager Software at OD_{620}. The analysis with the Gen5™ Microplate Reader was conducted in triplicate for each plate.

Analysis of absorbance readings: Absorbance readings of the reference strains and clinical isolates collected from numerous experimentation dates were combined together to find the median and standard deviation for each sample. The median for the samples were then used in two-tailed t tests where the absorbance reading of wild-type strain 8325-4 was compared with other sample absorbance readings. In this study, a p-value < 0.05 was considered to be significant.

RESULTS

When *S. aureus* Negative (ΔFnbpA and ΔFnbpB) was originally tested with clinical isolates and the wild-type of *S. aureus* (8325-4), it produced higher absorbance readings than expected. In one experiment, the absorbance reading median at 620 nm for *S. aureus* Negative was 0.7280. This reading was higher than the *S. aureus* wild (8325-4) in the same experiment (0.6950). High absorbance readings, such as the one observed with *S. aureus* Negative, indicate that more

cells were able to attach to the wells. In the human body, this translates to the idea of more *S. aureus* attaching to fibrinogen and increasing the chance of causing an infection such as IE. Absorbance readings, therefore, were indirectly measuring bond strength. Severity of IE and higher absorbance readings, on the other hand, have not been associated together in past studies, but it is an educated assumption that can be made. The original intention to use *S. aureus* Negative was to have a strain with an absorbance reading similar to the blank. Since it lacked the qualities that were needed, the *S. aureus* Negative was replaced by the Newman strains in later experiments. The Newman strains produce FnbpA and FnbpB; however, they are not expressed on the cell walls [6]. Therefore, the wild-type strain of Newman was a good reference to use to compare against the clinical isolates. Furthermore, the Newman strains that lacked either ClfA, ClfB, or both were good to understand the role of the clumping factors during the binding process to fibrinogen.

The median absorbance readings were determined for both clinical isolates and Newman strains from numerous experiments (Table 1). While apparent characteristics could be observed among the Newman strains, no distinct and clear association among clonal complexes or clinical isolates could be established from the data. The approach of taking absorbance readings for each sample and finding the median, however, yielded inconsistencies because of instrumental drift, and the absorbance readings for the same bacterial sample varied greatly depending on the experimentation day. Therefore, relative absorbance readings were also determined by using

absorbance readings of the Newman wild cells as the reference for each day. The median of the differences for each bacterial specimen were determined (Table 2). In Figure 3, the x-axis represents the absorbance reading of the wild Newman strain at 620 nm and the bars represent whether the absorbance reading for the sample strain was higher or lower than the reference strain. Wild-type Newman was chosen to be the standard because while it possesses ClfA and ClfB, they do not have FnbpA and FnbpB expressed on their cell walls [6]. It was the ideal strain to use when interpreting the binding characteristics of each of the clinical isolates because before any analysis, it was believed that fibronectin binding proteins were more important during the binding process.

Two-tailed t tests between absorbance readings of each bacterial specimen and the *S. aureus* wild (8325-4) were also conducted (Table 3). The wild-type strain *S. aureus* (8325-4) was used for the t tests instead of Newman wild because many previous studies have used this strain as a reference strain due to its low number of mutations and for commonly existing in natural conditions. Moreover, wild strain 8325-4 express all four MSCRAMMs (FnbpA, FnbpB, ClfA, and ClfB) examined in the experiment [2 and 12]. Comparing the Newman strains and clinical isolates with *S. aureus* wild (8325-4) would convey whether the compared specimen had a mechanism that allowed them to bind more to fibrinogen or a mechanism that prevented them from binding to fibrinogen. Absorbance readings of all samples except Newman wild and Newman ΔClfB were determined to be significantly different from reference absorbance readings (Table 3).

DISCUSSION

S. aureus is known to cause various infections within the human body and infective endocarditis (IE) is one of the many *S. aureus* infections that could be life-threating. Although the number of IE cases caused by *S. aureus* have been increasing over the past couple of decades, little is still known about how these microbes are able to bind inside the human body to cause infections. One theory is that IE is linked to the binding associated with MSCRAMMs on *S. aureus*. This study focused on fibronectin binding proteins A and B as well as clumping factors A and B.

One apparent observation with the Newman strains was that a lower absorbance reading was obtained for Newman ΔClfA strains than for Newman ΔClfB strains (Figure 4). This indicates that ClfA is more important for binding to fibrinogen than ClfB, at least for this reference strain. This difference between Newman ΔClfA and Newman ΔClfB is supported by findings from previous experiments, which indicate that ClfA has a greater role than ClfB when binding [2 and 9]. Furthermore, these past works show that ClfA masks the binding through ClfB [2 and 9]. Newman ΔClfA lacks ClfA so it would, in theory, have diminished binding affinity to fibrinogen than a sample that only lacks ClfB. The difference between these two clumping factors lies within the A domains and this may explain why ClfA has a greater effect when binding than ClfB.

Both ClfA and ClfB have similar structures in terms of signal sequence and wall attachment domains; however, only 26% of their A domains overlap [2]. The specific amino acids

that are responsible for making the effects of ClfA more significant are unknown, but this observation suggests that if the limited but existing similarities of both clumping factors can be targeted, both ClfA and ClfB will be prevented from functioning simultaneously.

Other observations from the experiment with the Newman strains can also be used to explain the relationship between ClfA and ClfB. Figure 4 suggests that the absorbance reading for Newman ΔClfA and Newman ΔClfB approximately adds up to the absorbance reading of the wild-type Newman strain. Figure 3, on the other hand, portrays the same concept from a different perspective. In this graph, the sum of the relative absorbance reading for Newman ΔClfA and the relative absorbance reading for Newman ΔClfB is approximately the same as the relative absorbance reading for Newman ΔClfA and B. These two observations point out that although ClfA has more significance during the binding process, both ClfA and ClfB are necessary MSCRAMMs for *S. aureus* to successfully bind to fibrinogen.

The results for the clinical isolates were more challenging to interpret (Figure 5). Unlike Newman strains that lack fibronectin binding proteins on their walls, these isolates have both fibronectin binding proteins as well as clumping factor proteins (Figure 6). Figure 3 provides an interesting comparison between the Newman strains and the clinical isolates. In this figure, the positive bars convey that the sample being compared has a higher absorbance reading than Newman wild while the negative bars state the opposite. With the exception of isolate #289 (CC5

CDC), all clinical isolates, including 8325-4, had positive values while all Newman strains had negative values.

This graphical characteristic suggests that all four MSCRAMMs examined in this study (FnbpA, FnbpB, ClfA, and ClfB) are necessary when binding to fibrinogen. While ClfA and ClfB are expressed in Newman strains and clinical samples, the Newman strains do not have FnbpA and FnbpB anchored on their cell walls [6]. Enhanced binding for most of the clinical isolates, which have *fnbA* and *fnbB*, suggests these two proteins are also critical for Fg-binding (Figure 6). Newman strains do in fact have both *fnbA* and *fnbB*; however, they have a nonsense mutation that results in the truncation of both Fnbps at the end of the C domain at identical locations. Previous experiments show that the truncated Fnbps are completely secreted into the culture medium and do not anchor to the cell walls of *S. aureus* since they lack the sortase motif (LPETG) [6].

When this relationship between the MSCRAMMs is viewed from a larger perspective, the same concept applies. Past studies point out that both fibronectin binding proteins and clumping factors are critical to initiate endocarditis [10]. Experimentation results have showed that both promote valve colonization, but it was also discovered that they have different roles during the colonization process. While ClfA promotes only early valve colonization, FnbpA is responsible for promoting both colonization and persistence, causing clinical symptoms commonly seen with *S. aureus* infections [10]. Even in the clinical aspect, both Fnbps and Clfs are critical components.

It is worth mentioning that the A domains of FnbpA and ClfA of *S. aureus* wild (8325-4) have ~ 24.8% amino acid identity and have ~ 46% amino acid similarity in the Fg binding regions. In addition, FnbpA and ClfB from the same sample have ~ 27.8% amino acid identity while they have ~ 49% amino acid similarity in the Fg binding regions [13]. These observations suggest that fibronectin binding proteins have similar structures to clumping factors, but also have additional structures not observed in clumping factors that promote binding to fibrinogen and are necessary to have a successful binding.

From a different approach, the clinical isolates also suggest an interesting point about themselves. In a two-sample t test where the clinical isolates were separated into two groups for analysis, nasal isolates (CDC) vs. bloodstream isolates (CDI and CDU), it was determined that there was significant difference between the two groups ($p = 0.0003117$). The grouping suggests that the origin of the *S. aureus* sample is critical. CDC are samples collected from the nasal cavity of patients with *S. aureus* while both CDI and CDU are samples collected from the bloodstream of patients with *S. aureus*. The result from the two-sample t test suggests that *S. aureus* that primarily resides in the nasal cavity (CDC) has a lower binding affinity to fibrinogen than *S. aureus* that is present in the bloodstream.

One possible reason for this result is due to the existence of various ligands in the nasal cavity in which *S. aureus* can bind to. The uppermost layer of epidermis of the nasal cavity is

composed of *stratum corneum* and this layer contains keratinocytes that express numerous proteins like loricrin, cytokeratin 10, and involucrin. Out of the many proteins expressed in the nasal cavity, loricrin and cytokeratin 10 are two ligands that ClfB frequently binds to [11]. This suggests that binding to fibrinogen may not be important for nasal isolates of *S. aureus*. and *S. aureus* classified as CDC may have more ClfB expressed on their cell walls rather than the other MSCRAMMs because ClfB is necessary for nasal colonization.

Furthermore, the lower binding affinity towards fibrinogen can be explained here too because, as mentioned in an earlier analysis with Newman strains, ClfA contributes more greatly towards the binding to fibrinogen than ClfB. If it is assumed that CDC strains have more ClfB expressed than ClfA, it makes sense to make the assumption that nasal isolates cannot bind as efficiently as bloodstream isolates to fibrinogen due to lower ClfA count. On the other hand, the reason why CDU and CDI strains had higher binding affinity to fibrinogen than CDC strains could be due to high count of ClfA. High ClfA count can be assumed since both CDU and CDI strains are samples from the bloodstream and blood has high concentrations of complement factor I and fibrinogen – two proteins that ClfA binds to [3]. To survive in the bloodstream and successfully cause infections, ClfA would therefore be necessary. Further research must be conducted to conclude that there are more ClfA counts on CDU and CDI strains while there are more ClfB counts on CDC with Western blotting and ELISA, but for now, it is the best conclusion to make in

regard to why CDC strains had lower binding affinity toward fibrinogen than CDI and CDU.

From the Newman strains, the role in which the clumping factors play during the binding process became apparent. As previous studies had suggested, ClfA had a more significant role during the binding process to fibrinogen, but ClfB was also necessary. In addition, the Newman strains also suggested that fibronectin binding proteins may be equally important during binding. On the other hand, little could be concluded about the clinical isolates. Although samples belonging in the same clonal complex had identical sequences for all four MSCRAMMs experimented, binding affinity to fibrinogen varied greatly and had no apparent correlations. There was, however, significant difference in binding affinity between isolates from the anterior nares and bloodstream. CDC strains did not bind as much as the other two clinical isolates and this was associated with the difference in types of ligand available. Further research must be conducted to interpret what type of MSCRAMM are expressed on each clinical isolate type as well as how much are expressed for each type. Moreover, how physical stress affects each MSCRAMM should also be examined since it is important to understand how these MSCRAMM tested in this study help *S. aureus* in an environment where blood will be constantly be flowing. The findings made in the current study, however, can be utilized in the near future for faster intervention toward people who are at risk of developing IE. Patients with artificial valves and *S. aureus* bacteremia should be treated as soon as possible to prevent possible IE development because compared to *S. aureus*

residing in the nasal cavity, ones present in the bloodstream will more likely bind to fibrinogen and would eventually develop into IE. On the other hand, treatment methods that target both clumping factors and fibronectin binding proteins concurrently is recommended when treating against IE. Both types of MSCRAMMs are factors that are equally important when preventing *S. aureus* from colonizing in the heart valve.

CONCLUSION

Infectious endocarditis caused by *S. aureus* is currently a growing public health concern throughout the world that can no longer be simply ignored. To make matters worse, cases of IE caused by MRSA have also been steadily growing. *S. aureus* infections, especially IE, are preventable and last resorts such as surgeries can be avoided if proper measures are taken ahead of time. In order to create prevention measures, the structures of each MSCRAMM expressed on *S. aureus* along with their roles during the binding to Fg must be analyzed more thoroughly. Little about *S. aureus* is known to the scientific world today, but with the future experiments on various aspects of their binding mechanism, more preventative public health measures can be taken towards vulnerable populations and towards patients already suffering from IE in the future.

Figure 1: Illustration of (a) FnbpA/B and (b) ClfA/B from *S. aureus* Newman. The N-terminus has a signal sequence and is followed by the A domain. For both MSCRAMM, the A domains bind to fibrinogen. The repeat region in FnbpA/B that follows allows *S. aureus* to bind to fibronectin. On the other hand, ClfA/B has a Ser-Asp repeat region. The C-terminus for both have a proline-rich repeat (PRR), wall (W), and membrane (M)-spanning domains. The numbers indicate which amino acid each domain begins in.

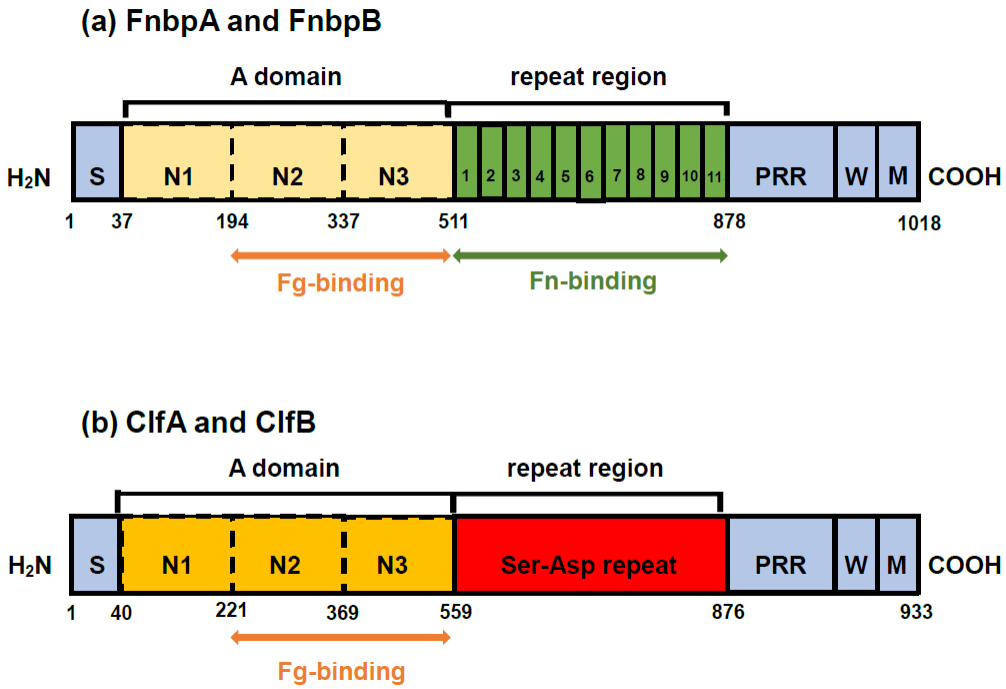


Figure 2: Amino acid sequence of FnbpA A domain and F1 region from 8325-4 and the clinical isolates. Red indicates the signal sequence, yellow shows the difference in amino acid with the reference 8325-4 strain, and blue is the insertion (ADD) or deletion (DEL) from the 8325-4.

S. aureus 8325 Adomain (FnbA)

MKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEAAASEQKTTT
VEENGNSATDNKTSETQTTATNVNHIEETQSYNATVTEQPSNAT
QVTTEEAPKAVQAPQTAQPANIETVKEEVVKEEAKPQVKETTQS
QDNSGDQRQVDLTPKKATQNQVAETQVEVAQPRTASESKPRVTR
SADVAEAKEASNAKVETGTDVTSKVTVEIGSIEGHNNTNKVEPH
AGQRAVLKYKLKFENGLHQGDYFDFTLSNNVNTHGVSTARKVPE
IKNGSVVMATGEVLEGGKIRYTFTNDIEDKVDVTAELEINLFID
PKTVQTNGNQTITSTLNEEQTSKELDVKYKDGIGNYYANLNGSI
ETFNKANNRFSHVAFIKPNNGKTTSVTVTGTLMKGSNQNGNQPK
VRIFEYLGNNEDIAKSVYANTTDTSKFKEVTSNMSGNLNLQNNG
SYSLNIENLDKTYVVHYDGEYLNGTDEVDFRTQMVGHPEQLYKY
YYDRGYTLTWDNGLVLYSNKANGNEKNGP

FnbA_CC8_CDC_172_Adomain (1AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSYNAT
VTEQPSNATQVTTEEAPKAVQAPQTAQPANIETVKEEVVKEEAK
PQVKETTQSQDNSGDQRQVDLTPKKATQNQVAETQVEVAQPRTA
SESKPRVTRSADVAEAKEASNAKVETGTDVTSKVTVEIGSIEGH
NNTNKVEPHAGQRAVLKYKLKFENGLHQGDYFDFTLSNNVNTHG
VSTARKVPEIKNGSVVMATGEVLEGGKIRYTFTNDIEDKVDVTA
ELEINLFIDPKTVQTNGNQTITSTLNEEQTSKELDVKYKDGIGN
YYANLNGSIETFNKANNRFSHVAFIKPNNGKTTSVTVTGTLMKG
SNQNGNQPKVRIFEYLGNNEDIAKSVYANTTDTSKFKEVTSNMS
GNLNLQNNGSYSLNIENLDKTYVVHYDGEYLNGTDEVDFRTQMV
GHPEQLYKYYYDRGYTLTWDNGLVLYSNKANGNGKNGP

FnbA_CC97_CDC_250_Adomain (120 AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKVNETQTTTNVNTIDETQSYSAT
ATEQPSNATQVTTEKAPKAVQAPQTAQPANVETVKEEVVKEEAN
PQVKETTQSQDNSGDQRQVDLTPKKATQNQAAETQVEVAQPRTV
SESKPRVTRSADVAEAKEASGAKVETGTDVTSKVTVESGSIEAP
QG(DEL)NKVEPHAGQRVVLKYKLKFEKGLHKGDYFDFTLSNNV
NTYGVSTARKVPEIKNGSVVMATGQLLGNGKIRYTFTDYIDYKV
NVTAQLEINLFIDPKTVQSNGQQTITSTLNDKETKNTLPIEYNP
GVSNSYANVNGSIETFDKGNNKFTHVAYIKPQNG(DEL)HKSDS
VSI(ADD)TGTLTQGSKANGNVPTVKVYEVLKDAKELPESVYAN
ISDSTMFKDVTQEMKDKLKVENNGSYKLDIEKLEKGYVIHYDGE
YLSGSDQVNFRTHMFGYPEQQYKYYYTHL(ADD)GYQLTWDNGL
VLYSNKAKGDGTNGT

FnbA_CC8_CDU_3450_Adomain (1AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSYNAT
VTEQPSNATQVTTEEAPKAVQAPQTAQPANIETVKEEVVKEEAK
PQVKETTQSQDNSGDQRQVDLTPKKATQNQVAETQVEVAQPRTA
SESKPRVTRSADVAEAKEASNAKVETGTDVTSKVTVEIGSIEGH
NNTNKVEPHAGQRAVLKYKLKFENGLHQGDYFDFTLSNNVNTHG
VSTARKVPEIKNGSVVMATGEVLEGGKIRYTFTNDIEDKVDVTA
ELEINLFIDPKTVQTNGNQTITSTLNEEQTSKELDVKYKDGIGN
YYANLNGSIETFNKANNRFSHVAFIKPNNGKTTSVTVTGTLMKG
SNQNGNQPKVRIFEYLGNNEDIAKSVYANTTDTSKFKEVTSNMS
GNLNLQNNGSYSLNIENLDKTYVVHYDGEYLNGTDEVDFRTQMV
GHPEQLYKYYYDRGYTLTWDNGLVLYSNKANGNGKNGP

FnbA_CC8_CDI_4495_Adomain (1AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSYNAT
VTEQPSNATQVTTEEAPKAVQAPQTAQPANIETVKEEVVKEEAK
PQVKETTQSQDNSGDQRQVDLTPKKATQNQVAETQVEVAQPRTA
SESKPRVTRSADVAEAKEASNAKVETGTDVTSKVTVEIGSIEGH
NNTNKVEPHAGQRAVLKYKLKFENGLHQGDYFDFTLSNNVNTHG
VSTARKVPEIKNGSVVMATGEVLEGGKIRYTFTNDIEDKVDVTA
ELEINLFIDPKTVQTNGNQTITSTLNEEQTSKELDVKYKDGIGN
YYANLNGSIETFNKANNRFSHVAFIKPNNGKTTSVTVTGTLMKG
SNQNGNQPKVRIFEYLGNNEDIAKSVYANTTDTSKFKEVTSNMS
GNLNLQNNGSYSLNIENLDKTYVVHYDGEYLNGTDEVDFRTQMV
GHPEQLYKYYYDRGYTLTWDNGLVLYSNKANGNGKNGP

FnbA_CC5_CDU_3854_Adomain (118AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSYNAT
VTEQPSNATQVTTEEAPKAVQAPQTAQPANVETVKE(DEL)EE(
DEL)KPQVKETTQPQDNSGNQRQVDLTPKKVTQNQGTETQVEVA
QPRTASESKPRVTRSADVAEAKEASDVSEVKGTDVTSKVTVESG
SIE(DEL)APQGNKVEPHAGQRVVLKYKLKFADGLKRGDYFDFT
LSNNVNTYGVSTARKVPEIKNGSVVMATGEILGNGNIRYTFTNE
IEHKVEVTANLEINLFIDPKTVQSNGEQKITSKLNGEE(DEL)T
EKTIPVVYNPGVSNSYTNVNGSIETFNKESNKFTHIAYIKPMN(
ADD)GNQSNTVSVTGTLTEGSNLAGGQPTVKVYEYLGKKDELPQ
SVYANTSDTNK(ADD)FKDVTKEMNGKLSVQDNGSYSLNLDKLD
KTYVIHYTGEYLQGSDQVNFRTELYGYPERAYKS(ADD)YYVYG
GYRLTWDNGLVLYSNKADGNGKNGQ

FnbA_CC5_CDC_289_Adomain (118AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSYNAT
VTEQPSNATQVTTEEAPKAVQAPQTAQPANVETVKE(DEL)EE(
DEL)KPQVKETTQPQDNSGNQRQVDLTPKKVTQNQGTETQVEVA
QPRTASESKPRVTRSADVAEAKEASDVSEVKGTDVTSKVTVESG
SIE(DEL)APQGNKVEPHAGQRVVLKYKLKFADGLKRGDYFDFT
LSNNVNTYGVSTARKVPEIKNGSVVMATGEILGNGNIRYTFTNE
IEHKVEVTANLEINLFIDPKTVQSNGEQKITSKLNGEE(DEL)T
EKTIPVVYNPGVSNSYTNVNGSIETFNKESNKFTHIAYIKPMN(
ADD)GNQSNTVSVTGTLTEGSNLAGGQPTVKVYEYLGKKDELPQ
SVYANTSDTNK(ADD)FKDVTKEMNGKLSVQDNGSYSLNLDKLD
KTYVIHYTGEYLQGSDQVNFRTELYGYPERAYKS(ADD)YYVYG
GYRLTWDNGLVLYSNKADGNGKNGQ

FnbA_CC5_CDC_204_Adomain (118AA difference)

ILHLKGDIIVKNNLRYGIRKHKLGAASVFLGTMIVVGMGQDKEA
AASEQKTTTVEENGNSATDNKTSETQTTATNVNHIEETQSYNAT
VTEQPSNATQVTTEEAPKAVQAPQTAQPANVETVKE(DEL)EE(
DEL)KPQVKETTQPQDNSGNQRQVDLTPKKVTQNQGTETQVEVA
QPRTASESKPRVTRSADVAEAKEASDVSEVKGTDVTSKVTVESG
SIE(DEL)APQGNKVEPHAGQRVVLKYKLKFADGLKRGDYFDFT
LSNNVNTYGVSTARKVPEIKNGSVVMATGEILGNGNIRYTFTNE
IEHKVEVTANLEINLFIDPKTVQSNGEQKITSKLNGEE(DEL)T
EKTIPVVYNPGVSNSYTNVNGSIETFNKESNKFTHIAYIKPMN(
ADD)GNQSNTVSVTGTLTEGSNLAGGQPTVKVYEYLGKKDELPQ
SVYANTSDTNK(ADD)FKDVTKEMNGKLSVQDNGSYSLNLDKLD
KTYVIHYTGEYLQGSDQVNFRTELYGYPERAYKS(ADD)YYVYG
GYRLTWDNGLVLYSNKADGNGKNGQ

Table 1: The table contains the median absorbance readings at 620 nm for both clinical isolates and reference strains.

S. aureus sample	absorbance reading median at 620 nm
Newman ΔClfA	0.1955
Newman ΔClfB	0.5670
Newman ΔClfA ΔClfB	0.1410
Newman wild	0.5955
8325-4 (wild)	0.4845
250 (CDC - CC97)	0.5560
172 (CDC - CC8)	0.6125
289 (CDC - CC5)	0.7520
204 (CDC - CC5)	0.5200
3854 (CDU - CC5)	0.6470
3450 (CDU - CC8)	0.3300
4495 (CDI - CC8)	0.7455
blank	0.1315

Table 2: Table consists of median relative absorbance readings using wild-type *S. aureus Newman*. *S. aureus Newman* readings were subtracted from sample readings.

S. aureus sample	relative absorbance reading at 620 nm
Newman ΔClfA	-0.4125
Newman ΔClfB	-0.0555
Newman ΔClfA ΔClfB	-0.5100
172 (CDC - CC8)	0.0370
250 (CDC - CC97)	0.0295
289 (CDC - CC5)	-0.0770
3854 (CDU - CC5)	0.1255
4495 (CDI - CC8)	0.1365
8325-4 (wild)	0.1385

Table 3: Table with p-values obtained from two-tailed t tests conducted between median absorbance readings of *S. aureus* wild (8325-4) and absorbance readings of both clinical isolates and Newman strains. p-values lower than 0.05 are considered significant and are interpreted that there is a significant difference between absorbance readings of *S. aureus* wild (8325-4) and the compared *S. aureus* specimen.

S. aureus sample	p-value
Newman ΔClfA	2.8280E-8
Newman ΔClfB	0.1111
Newman ΔClfA ΔClfB	1.2700E-9
Newman wild	0.6689
250 (CDC - CC97)	1.7169E-7
172 (CDC - CC8)	6.5172E-5
289 (CDC - CC5)	2.7706E-6
204 (CDC - CC5)	0.0002
3854 (CDU - CC5)	1.8165E-6
3450 (CDU - CC8)	2.5060E-8
4495 (CDI - CC8)	0.0009

Figure 3: Relative absorbance reading using wild-type *S. aureus Newman* as the baseline. The sample and *S. Newman* came from the same column in the microtiter plate. The x-axis represents the wild-type *S. Newman*'s absorbance reading. The yellow bars represent strains that had a smaller absorbance reading than the reference strain while the blue bars represent strains that had higher absorbance readings than referenced Newman strain.

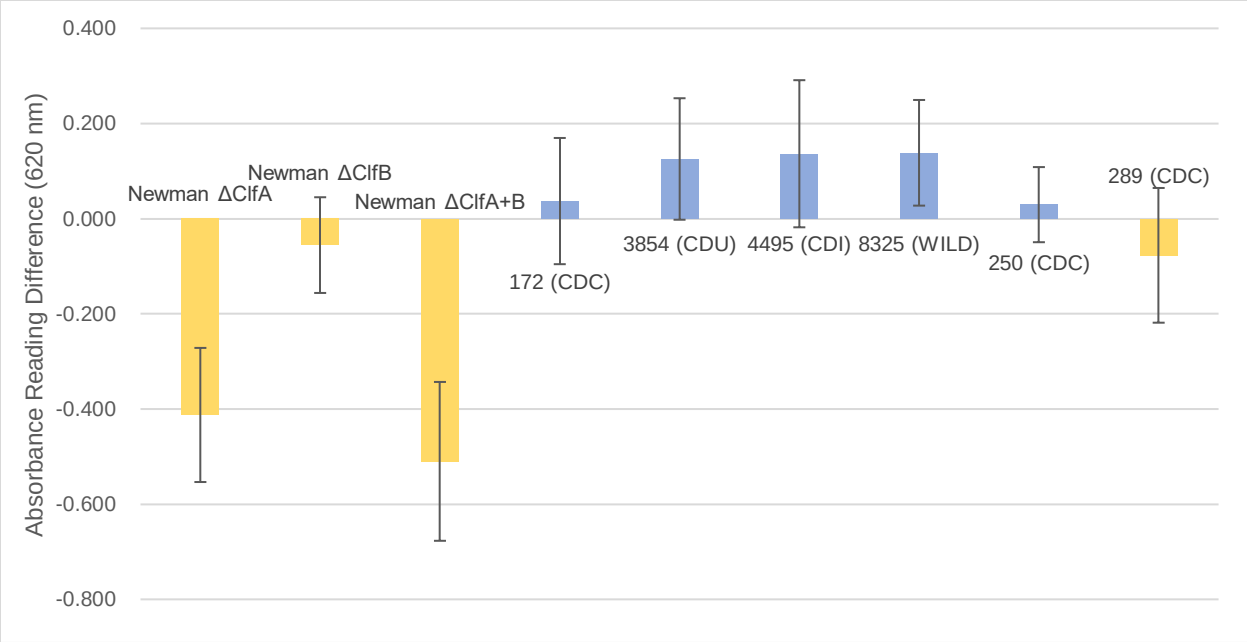


Figure 4: Graphical representation of the absorbance readings taken at 620 nm. While Newman ΔClfA and B have the lowest absorbance reading, Newman ΔClfA also has a very small absorbance reading when compared to Newman ΔClfB and Newman wild.

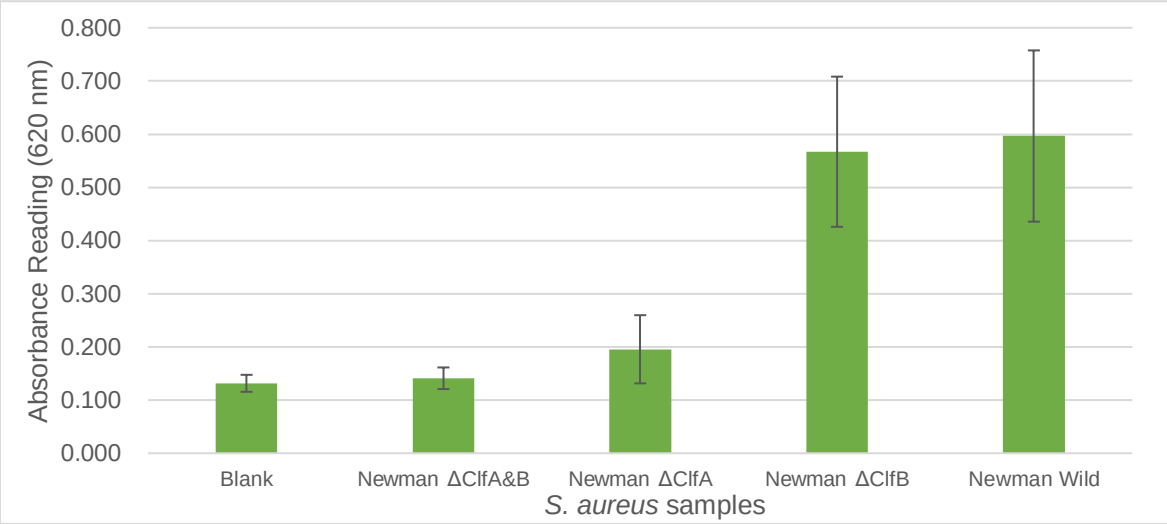
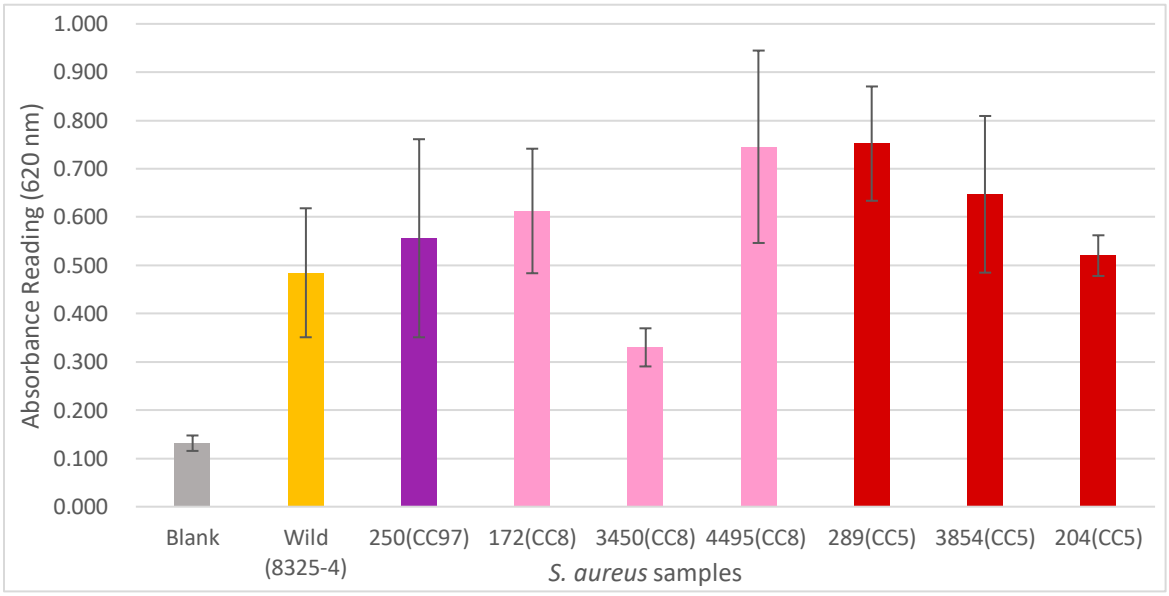


Figure 5: Graphical representation of absorbance readings of clinical isolates grouped by (a) bacterial lineage (i.e. clonal complexes) as well as (b) clinical isolate groups. The gray and golden yellow for both graphs represent the blank control and *S. aureus* wild (8325-4), respectively. In (a) purple represents clonal complex (CC) 97, pink is CC8, and red is CC5. For (b) light orange represents CDC, light green represents CDU, and light blue represents CDI.

(a)



(b)

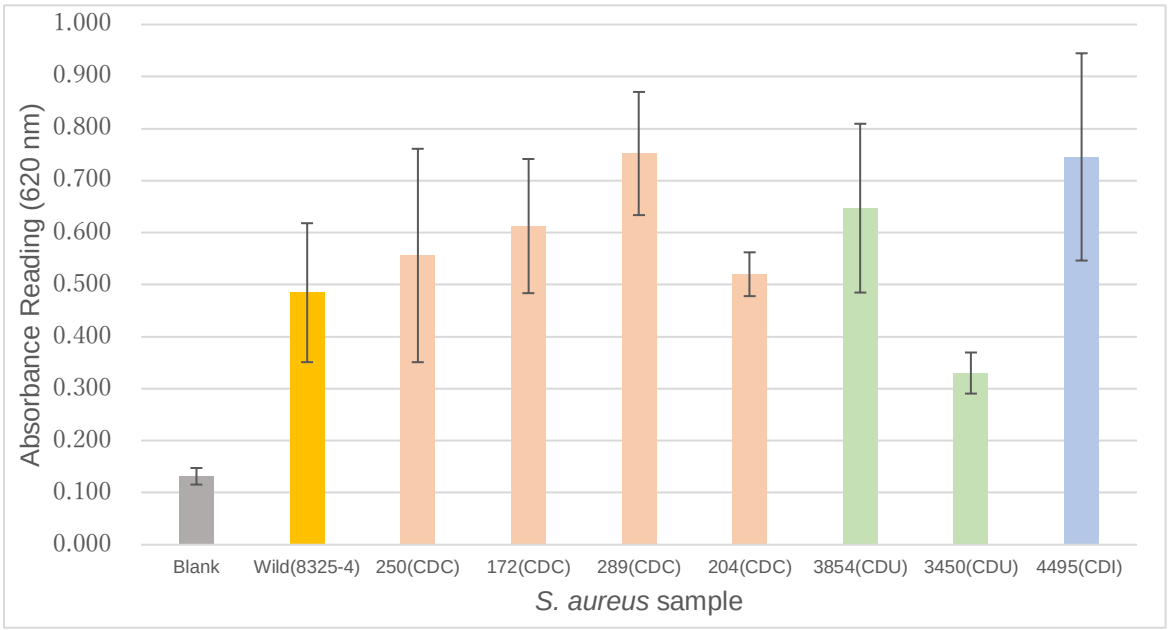
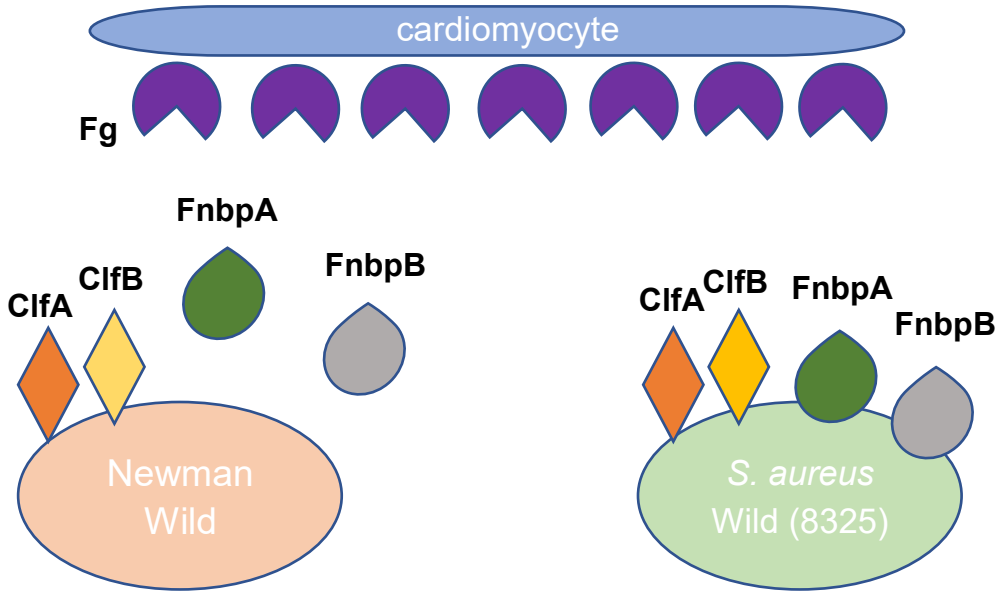


Figure 6: Schematic representation of MSCRAMM attached on the walls of *S. Newman* and *S. aureus* 8325-4. While both strains produce Fn-binding proteins and clumping factors, Newman does not express both types of MSCRAMM on their cell wall.



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