THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCES

# THE CLINICAL RELEVANCE OF HETEROGENEOUS β-LACTAM RESISTANCE IN CA-USA300

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SPRING 2014

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biology
with honors in Veterinary and Biomedical Sciences

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# ABSTRACT

In order for a strain of *Staphylococcus aureus* to be classified as MRSA, it must possess the gene *mecA* and its product, PBP2a. However, while mecA is essential for high levels of resistance to β-lactam antibiotics, it is not sufficient. In fact, previous studies have demonstrated that clinical isolates of community-associated USA300 can possess a wide variety of $MIC_{99}$ values between cells, ranging from 0.1-1000 μg/mL OX. However, only 0.1% of these cells account for resistance above 10 μg/mL OX range. This phenomenon of heterogeneous β-lactam resistance (HBLR), where a vast majority (99.9%) of cells in the same strain are weakly resistant and a small subpopulation are highly resistant, may explain why clinical treatments of CA-MRSA strains such as USA300 with β-lactams are typically ineffective. While HBLR has been demonstrated to occur through various *in vitro* experiments, very little work has been done to show that it can occur *in vivo* and thus is clinically relevant.

In this study, we subjected a simulated endocardial vegetation of USA300 to a 72-hour oxacillin treatment to replicate the *in vivo* treatment of endocarditis in a patient, hypothesizing that the final vegetation would primarily consist of bacterial cells with greater β-lactam resistance than the wild-type or initial inoculum. Colonies isolated from the vegetation after this 72 hour period were characterized in contrast to wild-type USA300. Multiple analyses on the resistance and fitness levels of these samples confirmed that the colonies present after the *in vivo* simulation were significantly more resistant to β-lactams than wild-type USA300. In addition, genomic analyses revealed these isolates were part of a unique subpopulation of spontaneous mutants, ultimately supporting that HBLR is a clinically relevant phenomenon.

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# ACKNOWLEDGEMENTS

First of all, I would like to thank Dr. Michael Mwangi for giving me the opportunity to join his lab and, in the process, introducing me to a field of research that I will continue exploring in graduate school and throughout the rest of my academic and professional career. My deepest gratitude also goes out to Caitlin Grube, Vikas Koundal, and Juan Antonio Raygoza, who took the time to teach me the basic laboratory techniques necessary for work with MRSA and always made themselves available for help and advice throughout the many phases of this project. I would also like to thank Dr. Lester Griel for his guidance and insight throughout this process. Finally, I would like to thank Dr. Michael Rybak, Dr. Lu Bai, and Chao Yan for their help with data collection, enabling me to bring my research to a higher-level while still an undergraduate.

# CHAPTER 1

## Introduction

Since its first description in 1961, methicillin resistant *Staphylococcus aureus*, more commonly known as MRSA, has become a global pathogen, causing a variety of human diseases from skin infections to septicemia and toxic shock (Garcia-Alvarez, Holden et al. 2011). This has been largely attributed to the widespread emergence of CA-MRSA in healthy patients who have not recently experienced any other infection or disease (Bancroft 2007). One of the most prominent strains of CA-MRSA now facing the United States is USA300, which has also been associated with a multitude of skin infections throughout the world, with noted incidents in Canada, the Middle East, and 15 European countries since 2000 (Diep, Gill et al. 2006, Nimmo 2012).As a testament to its virulent ability, it is now the source of over 60% of all clinical outbreaks of *S. aureus* treated in hospital intensive care units in the United States (Pasquale, Jabrocki et al. 2013).

The standard treatment for a *S. aureus* infection has been through β-lactams, antibiotics that are able to interact with penicillin binding proteins (PBPs) that are essential for cell wall synthesis (Kohanski, Dwyer et al. 2007, Bazan, Martin et al. 2011). Specifically, β-lactams such as penicillin are proposed to interact with PBP2, inhibiting the transpeptidase activity that links the murein glycopeptide subunits in peptidoglycan that are necessary for a functional cell wall. In an actively dividing bacterium, these unlinked subunits would be integrated into the cell wall and compromise the cell's ability to tolerate osmotic pressure from the cytoplasm, ultimately leading to cell lysis and death. As such, penicillin and other β-lactams are only effective in actively

growing cultures of cells since they can only affect newly synthesized peptidoglycan and cell walls, not pre-existing molecules (Tomasz 1979).

In the wake of this penicillin-driven selection, strains of *S. aureus* possessing penicillinases became more prominent, diminishing the drug's ability to treat infections (Tomasz 1979). The subsequent rise of methicillin as the first penicillinase-resistant β-lactam, however, has led to the current prominence of methicillin (or, in some cases, multidrug) resistance in *S. aureus* as conferred by *mecA* (Lowy 2003).

The gene *mecA* encodes a unique penicillin binding protein known as PBP2a, which has significantly lower affinity to β-lactams than its counterpart, PBP2. As such, β-lactams such as methicillin are unable to bind to PBP2a, which can then still perform its transpeptidase activity in order to continue cell wall synthesis, even in the presence of antibiotic (Berger-Bachi and Rohrer 2002). Although it has previously been demonstrated that *mecA* and PBP2a are necessary for high levels of resistance to β-lactams, there is no direct correlation between the extent of *mecA* expression and the $MIC_{99}$ for a particular strain of bacteria. This discovery has led to the idea that there are factors apart from *mecA* that can account for high levels of antibiotic resistance (Nakao, Imai et al. 2000).

While it is known, for instance, that environmental conditions such as reduced temperature or increased salt concentration can lead to higher levels of resistance, the exact mechanisms by which this resistance arises is unknown. Some theories detail that higher temperatures (37°C or greater) elicit heat shock responses in bacteria, inducing transcriptional changes that may result in the activation or inhibition of factors apart from *mecA* that contribute to resistance (Sieradzki, Chung et al. 2008).

Even under normal growth conditions, however, it has been demonstrated that cells within the same strain of CA-MRSA can possess a wide variety of $MIC_{99}$ values to β-lactams. In the case of *S. aureus*, cells within an individual clinical sample may possess a $MIC_{99}$ range of 6.3

– 1600 μg/mL ME. Perhaps the most noteworthy facet of this heterogeneous range of resistances is that a vast majority of the cells will possess weak levels of resistance to an antibiotic, whereas $1:10^4$ or $1:10^6$ will demonstrate high resistance (Fujimura and Murakami 1997). In the case of various strains of *S. aureus*, 99.9% of all bacterial cells with be killed by a concentration of 10 μg/mL OX or less, whereas 0.1 – 1% of cells will possess resistance to 10 μg/mL OX or higher (Finan, Rosato et al. 2002).

It is hypothesized that these resistant cells are spontaneous chromosomal mutants, once they are selected for by antibiotic, the trait of resistance is heritable and breeds true even on plain media (Ender, Berger-Bachi et al. 2009). Studies have shown that such resistors will retain uniform antibiotic resistance and fail to revert to heterogeneously resistant culture even after 60 generations on antibiotic-free media (Finan, Rosato et al. 2002). One concern, however, in studies involving these resistors is the presence of other bacterial cells known as tolerators or persistors. Tolerators are bacteria that have a heritable trait of resistance and are capable of surviving antibiotic treatment, but are unable to grow before the drug degrades. Persistors, however, are cells that remain dormant or simply halt growth in the presence of antibiotic to survive treatment, but do not have a heritable trait of resistance (Orman and Brynildsen 2013). In order to properly distinguish between these groups of cells, it is necessary to directly observe cell growth in the presence of an intact antibiotic through the use of time-lapse microscopy.

This phenomenon, known as heterogeneous β-lactam resistance (HBLR), in which a vast majority of cells are weakly resistant to β-lactam antibiotics while a small subpopulation is strongly resistant may explain why many β-lactam treatments of clinical MRSA infections are ineffective and result in high mortality and morbidity rates for these diseases driven by *S. aureus*, such as a 47% mortality rate for staphylococcal-linked endocarditis due to a lack of viable treatment options (Tsuji and Rybak 2005). When a β-lactam is used to treat a USA300 infection, it may simply remove the 99.9% of weakly resistant cells and remove any competition for the

strongly resistant subpopulation, enabling it to prolong the disease and cause further harm to the patient (Ender, Berger-Bachi et al. 2009).

While there have been multiple previous studies demonstrating that heterogeneity among USA300 and other MRSA strains can occur, these have all focused on *in vitro* experiments. In order to demonstrate the clinical relevance of HBLR and support that it is a noteworthy medical concern, it is necessary to show the presence of a heterogeneously resistant population of cells through an *in vivo* model.

The primary goal of this project is to better characterize HBLR in USA300 as a clinically relevant phenomenon by analyzing clinical isolates of USA300 following antibiotic treatment in an *in vivo* simulation to verify the existence of highly resistant subpopulation of spontaneous mutant cells in a clinical setting. This will be generated under the assumption that any colonies isolated from a vegetation after 72 hours of antibiotic treatment will possess significantly greater resistance levels to β-lactams and other antibiotics involved in the inhibition of cell wall synthesis than a wild-type sample of the strain. In addition, the fitness cost associated with increased resistance will be determined by doubling time estimations of the mutant cells along with the wild-type, and the presence of unique mutations in the resistors compared to the wild-type samples will be determined.

# CHAPTER 2

## Materials and Methods

### Preparation of Simulated Endocardial Vegetations (SEVs)[1]

Samples of *Staphylococcus aureus* strain USA300 purchased from the American Type Culture Collection (ATCC) were originally obtained from the skin abscess of a HIV patient. Vegetations for use in the *in vitro* model were generated by combining 0.25-1.0 mL of human cryoprecipitate, 0.1 mL of a USA300-inoculated MHB tube, 0.025 mL of platelets mixed with normal saline (Hershberger, Coyle et al. 2000) in a 1.5 mL Eppendorf. 5,000 U bovine thrombin was also added to each tube after inserting a monofilament line.

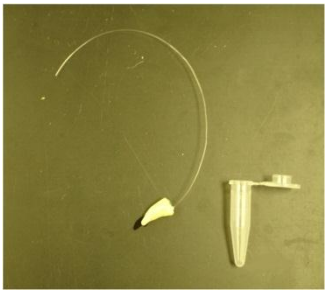


**Figure 1: A Sample SEV with a Monofilament Line**

### *In Vitro* Pharmokinetic/Pharmodynamic Simulated Endocardial Vegetation (PK/PD SEV) Model[1]

A two-compartment glass apparatus (modeling the heart) was filled with 250 mL MHB, and the prepared SEVs suspended from ports using the monofilament lines. To simulate *in vivo* conditions of the heart, the apparatus was incubated in a 37°C water bath for the duration of the experiment (Hershberger, Coyle et al. 2000). Oxacillin in the form of 1-2 g oxacillin sodium was injected into the model through another port every 4 hours, generating a clinically realistic fluctuation in antibiotic concentration (Baxter Healthcare Corporation, 2012). A magnetic

**Figure 2: The Two-Compartment Glass Apparatus used for the PK/PD SEV Model**

[1] The following work was performed by the Michael Rybak group at Wayne State University.

stir bar in the bottom of the apparatus ensured proper mixing of the media and antibiotic, and peristaltic pumps were used to remove media and antibiotic while additional media was constantly provided through separate ports in the apparatus (Hershberger, Coyle et al. 2000). The model was run for a period of 3 days, over which time one SEV was removed at the 0, 12, 24, 36, 48, 60, and 72 hour time points. A swab of each SEV was then streaked to form a culture on an individual TSA plate. Table 1 describes the contents of the two plates focused on in this experiment.

| Plate ID | Strain | Plate Contents |
|---|---|---|
| $t_0$A1 | USA300 | SEV extracted from the model after 0 hours |
| $t_{72}$A1 | USA300 | SEV extracted from the model after 72 hours |

**Table 1: SEV Streak Plate Identification**

While the SEV model is not a true *in vivo* experiment, and thus does not account for the host defense mechanism present in the more typical rabbit model, a previous study by Rybak has shown there is no significant difference between the results obtained from the two models. (Hershberger, Coyle et al. 2000).

**Mutant Isolation and Stock Preparation**

A bacterial suspension was generated for the $t_{72}$A1 plate by collecting three swaths of growth with separate sterile inoculating loops, and re-suspending each collection in the same tube containing 1 mL TSB. This mixture was serially diluted with additional TSB to create seven concentrations of the suspension ranging from $10^0$ to $10^{-6}$. 50 µL of each dilution was then spread for isolation on an individual TSA plate and grown for 72 hours at 37°C. 10 colonies were randomly picked from the $10^{-5}$ dilution plate and used to generate overnight cultures in 6 mL TSB incubated in a shaker at 37°C.

| Sample ID | Description |
|---|---|
| US300-WT | Wild-type USA300 |
| US300-C1 | Resistant colony of USA300 extracted from the $t_{72}$A1 plate spread for isolation |
| US300-C2 | |
| US300-C3 | |
| US300-C4 | |
| US300-C5 | |
| US300-C6 | |
| US300-C7 | |
| US300-C8 | |
| US300-C9 | |
| US300-C10 | |

**Table 2: SEV Colony Isolate Identification**

1 mL of each overnight culture was placed into its own 2 mL cryogenic vial along with 400 μL of 50% glycerol and vortexed to generate a frozen stock. These stocks were stored at -80°C while not in use. This procedure was repeated with the standardized USA300 wild-type to create an additional frozen stock.

**gDNA Extraction and Sequencing**

gDNA was extracted from a culture of a colony from the $t_0$A1 plate, USA300-C1, and USA300-C2 using an E.Z.N.A. Bacterial DNA Kit (Omega Bio-Tek, 2013). gDNA concentrations were quantified using a Qubit 2.0 Fluorometer and Nanodrop 2000 to reaffirm the samples' purity (Life Technologies Corporation, 2010, Thermo Fisher Scientific, 2009). Using the Qubit gDNA concentration values for each sample, 1000 ng gDNA was pipetted into a 0.2 mL PCR tube and sequenced at Genomics Core Facility as part of the Huck Institutes of the Life Sciences. Sequencing was performed in 500-cycle runs on an Illumina MiSeq, to prepare 2 x 250 bp paired-end reads (Illumina Inc., 2010). Template assemblies of reads were created using SeqMan NGen 4.1.2, and all three samples (both the $t_0$A1 colony and $t_{72}$A1 mutants) had their sequences aligned to the GenBank sequence for wild-type USA300. Mutations between the wild-type reference and the new sequences were identified using SeqMan Pro 10.1.2.

**Population Analysis Profiles**

Overnight cultures were prepared in test tubes containing 6 mL TSB from the frozen stocks of all 11 bacterial isolates. Each culture was then serially diluted with additional TSB to create a $10^{-6}$ dilution of a particular isolate. 50 μL was then spread for isolation on a series of 14 TSA plates with varying concentrations of oxacillin based on a $\log_2$ scale, including 0, 0.125, 0.25... 128, 256, and 512 μg/mL OX. All plates were incubated at 37°C for 72 hours, and the number of CFUs per plate counted after 12, 24, 36, 48, 60, and 72 hours had passed. These numbers were used to determine the OX $MIC_{99}$ of each strain and, in turn, distinguish the highly

resistant subpopulation from the initial heterogeneous wild-type isolate (Mwangi, Kim et al. 2013).

**Doubling Time Estimation**

The doubling time for each bacterial isolate was estimated by growing overnight cultures in tubes of 6 mL TSB at 37°C for the 10 resistant mutants along with three replicates of US300-WT. An appropriate amount of culture was added to a flask (or Flask #1) containing 50 mL TSB to generate an $OD_{600}$ of 0.005 and restore the bacterial population from stationary phase to the lag phase of growth. Flask #1 was incubated at 37°C until reaching a secondary $OD_{600}$ of 0.25 - 0.5. 1020 μL of this culture was then added to another flask (Flask #2) containing 50 mL TSB in order to generate a 1:50 bacterial dilution, this time escaping the lag phase of growth. Flask #2 was incubated at 37°C until also reaching a tertiary $OD_{600}$ of 0.25 – 0.5. The start and stop times of the growth period were recorded and used in conjunction with the secondary and tertiary $OD_{600}$ values to calculate the approximate doubling time for each sample.

All flasks used in the experiment were incubated and shaken at 37°C before inoculation in order to provide a similar environment for bacteria and prevent the onset of lag phase.

**E-Test Application and Cross-Resistance Evaluations**

E-Tests for the following 11 antibiotics were applied to each of the 11 bacterial isolates to gauge cross-resistance: oxacillin, benzylpencillin, cefuroxime, ceftaroline, bacitracin, fosfomycin, vancomycin, ciprofloxacin, rifampicin, linezolid, and daptomycin. Tests were performed in accordance to both the CLSI and preferred growth guidelines in order to gauge the effect of the environment on resistance levels. A sterile cotton swab was used to spread a $10^{-2}$ dilution in TSB of an isolate overnight culture on to an agar plate and incubated for growth for 72 hours before reading the final result. CLSI growth plates were conducted on MHA with 2% NaCl at 35°C, whereas preferred growth plates were conducted on TSA at 37°C (BioMérieux 2012).

The CLSI guidelines are the standard growth conditions for MRSA utilized by the Clinical Laboratory Standards Institute in order to determine resistance levels, whereas the preferred guidelines are the conditions primarily utilized by the Michael Mwangi group to cultivate USA300. The preferred guidelines are incorporated into this experiment to determine if an alternative environment will yield a significant difference in the USA300 $MIC_{99}$ values.

**Time-Lapse Microscopy**

Overnight cultures of US300-WT and US300-C1 were prepared in 6 mL tubes of TSB. 25 µL of each culture was pipetted into a sterile spectrometer tube with 2 mL TSB and incubated in a shaker at 37°C until reaching an $OD_{660}$ of .100-.200.

During this time, two agarose gel solutions were prepared by mixing 75 mg quick-melting agarose and 5 mL sterile TSB in a test tube and microwaving the contents for periods of 5 seconds at a time until the agarose fully dissolved. 2 µL of 0 µg/mL OX liquid stock was added to one tube and 2 µL of 25,000 µg/mL OX liquid stock to the other in order to respectively generate a 0 µg/mL OX and 10 µg/mL OX agarose gel solution. 2 mL of each solution was evenly pipetted onto its own 22x60mm coverslip and given time to solidify. Each gel was divided into small squares using a sterile razor blade.

At this time, the two grown USA300 cultures were sonicated to separate bacterial cells, and 1 µL of each culture was used to inoculate a square of the 0 µg/mL OX gel as well as a 10 µg/mL OX gel square. This culminated in 4 gel squares in total, which were then inverted and placed on a new 22x60mm coverslip and partially sealed with Vaseline to diminish gel dehydration and shrinkage during a time-lapse video. Three bacterial cells from each square had its growth then recorded over a period of 8 hours, with one photo being taken every 3 minutes, using the Bai Group phase-contrast microscope.

This entire procedure was repeated for the US300-WT and US300-C2 isolates.

# CHAPTER 3

## Results

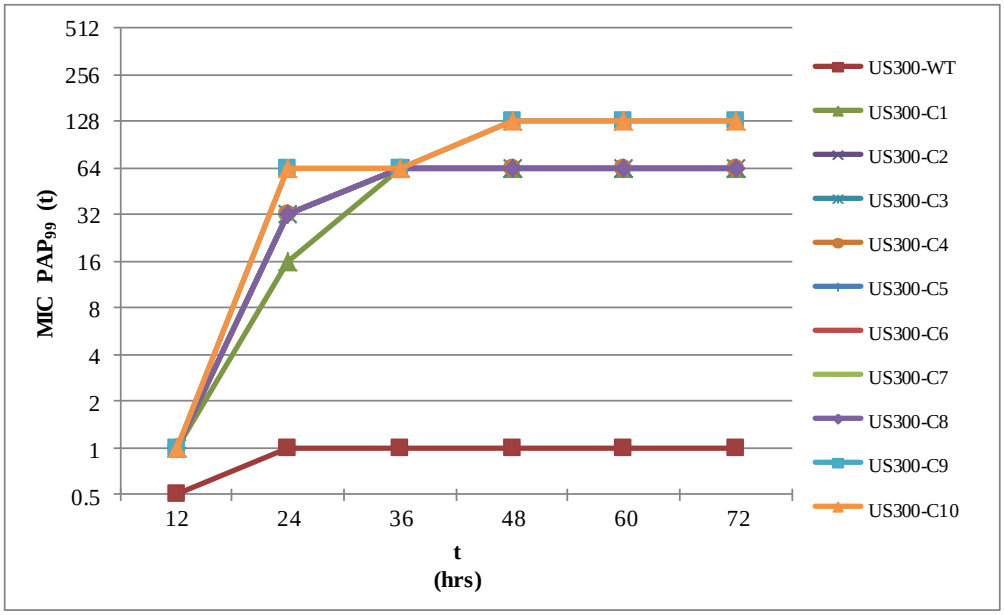



**Figure 3: MIC $PAP_{99}$ of the WT and Mutant USA300 Isolates on TSA over Time**. Figure 3 displays the $MIC_{99}$of the set of plates in each PAP at a given time point, or MIC $PAP_{99}$. Over a period of 72 hours, the US300-WT sample only reaches a maximum $MIC_{99}$ of 1 µg/mL OX. This is in stark contrast to all of the mutant isolates, which have an initial $MIC_{99}$ of 1µg/mL OX and ultimately reach resistance to 64 or 128 µg/mL OX by the end of the experimental run. After 12 hours of growth there is a noticeable difference between the resistance levels of the mutants in comparison to the wild-type sample.

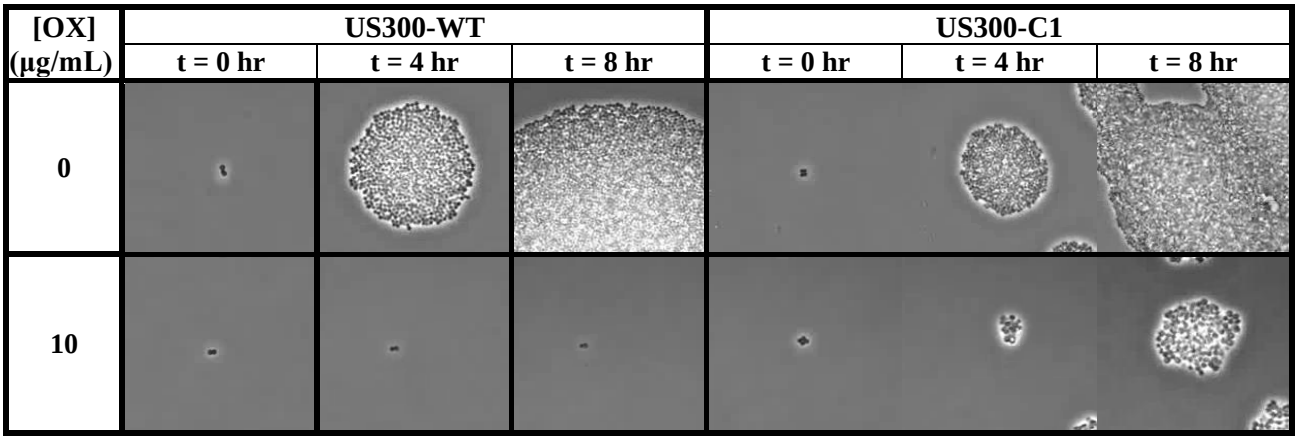



**Figure 4: Phase-Contrast Time Lapse Microscope Images of US300-WT and US300-C1 Growth on 0 and 10 μg/mL OX Agar over Time.** A single cell was identified from each of the phase contrast time-lapse videos of US300-WT and US300-C1 in 0 and 10 μg/mL OX media and its growth represented at 0, 4, 8 hr. It appears that US300-WT and US300-C1 are both capable of normal growth in antibiotic-free media, though US300-C1 demonstrates a slower growth rate after 4 hr. In the presence of 10 μg/mL OX, however, the wild-type cell demonstrates no growth, whereas the mutant grows, albeit less quickly than in the absence of oxacillin.

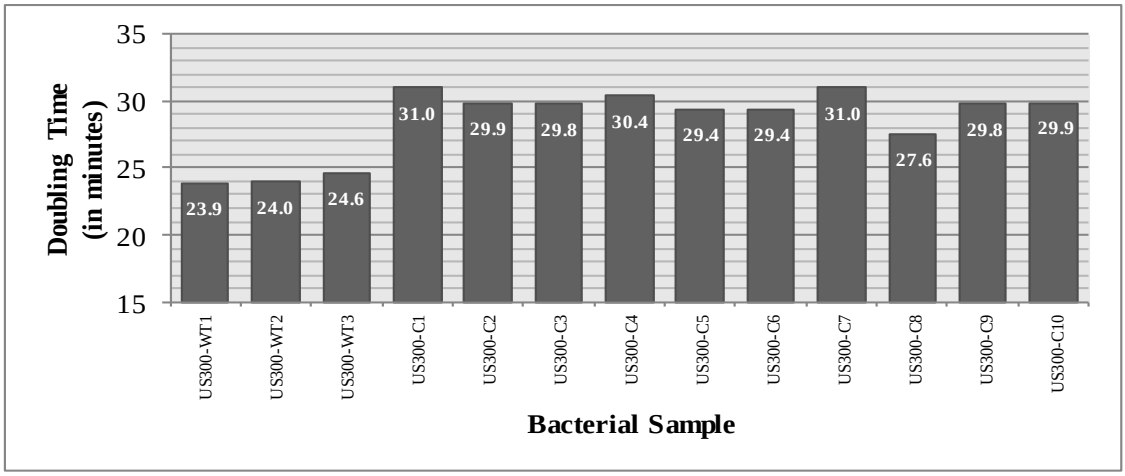



**Figure 5: Estimated Doubling Times of USA300 Wild-Type and Mutant Colony Isolates.**

The doubling time in the three replicates of US300-WT ranges from 23.9-24.6 minutes, where there is a significantly larger doubling time in all of the mutants samples, ranging from 27.6-31.0 minutes. All doubling times were calculated by using the following equation.

$$\mathbf{D_t = \frac{(t_2\text{-}t_1)}{\log_2(O_3/O_2 \times 50)}}$$

Where:

**$D_t$** = Doubling time (in minutes)

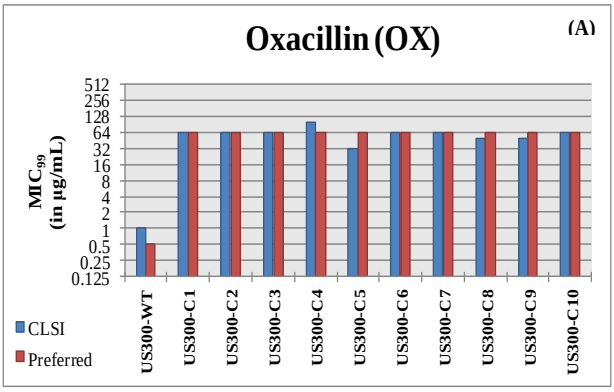
**$(t_2 – t_1)$** = Time (in minutes) for Flask #2 to grow to $OD_{600}$ of 0.25-0.500

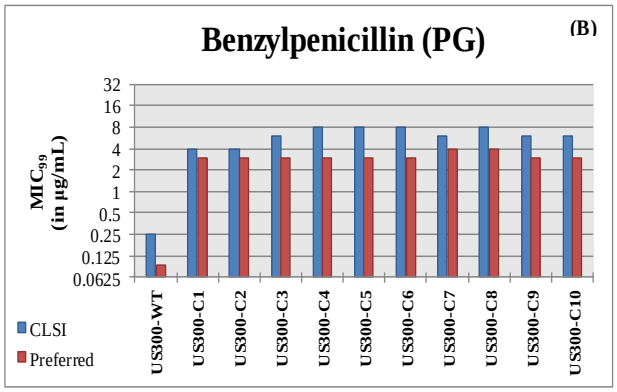
**$O_2$ =** Final $OD_{600}$ of Flask #1

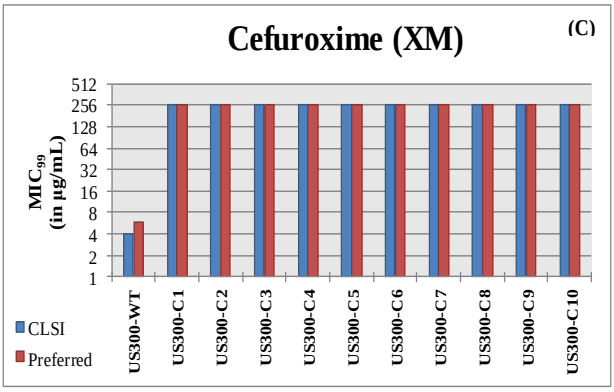
**$O_3$ =** Final $OD_{600}$ of Flask #2

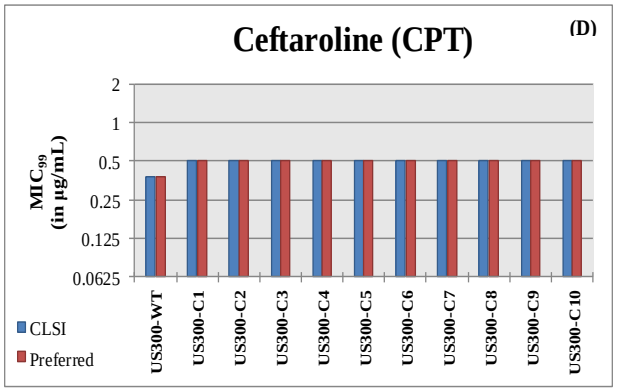
This equation was generated based on the principle of dividing the time elapsed in an experiment (or $t_2$-$t_1$) by the number of generations produced throughout the same interval of time. $O_3/O_2$ provides a comparison of the final $OD_{600}$ values for each flask, and is multiplied by 50 to take into account the fact that Flask #2 was the result of a 1:50 dilution from Flask #1. By taking the $\log_2$ of this ratio, it is possible to calculate how many generations (or doublings) occurred between the two flasks. As such, the equation as a whole will ultimately determine the doubling time, or the amount of time necessary for one generation to arise.

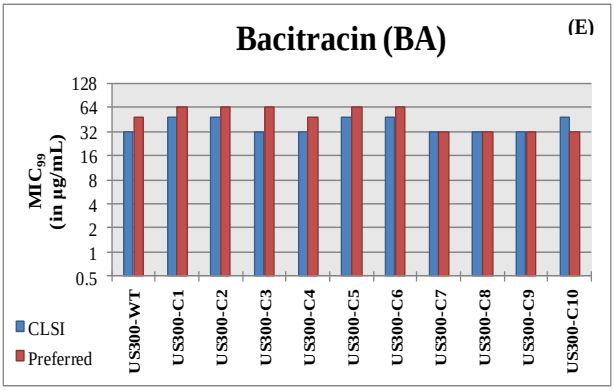
**Figures 6A-6K: E-Test $MIC_{99}$ Under CLSI and Preferred Growth Conditions.**

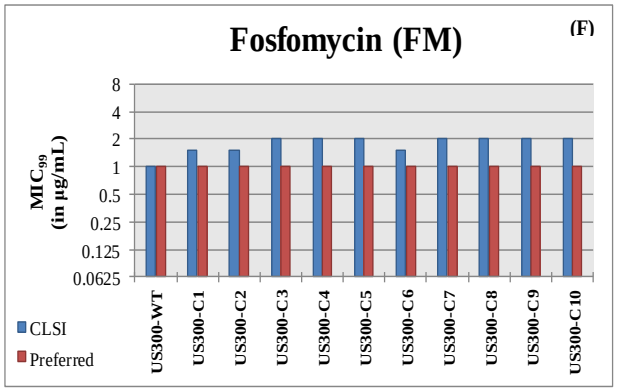


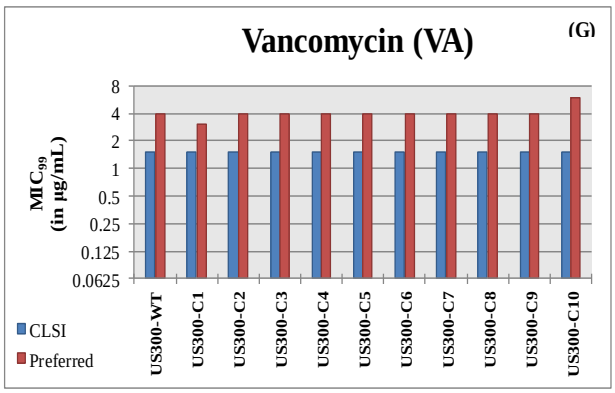



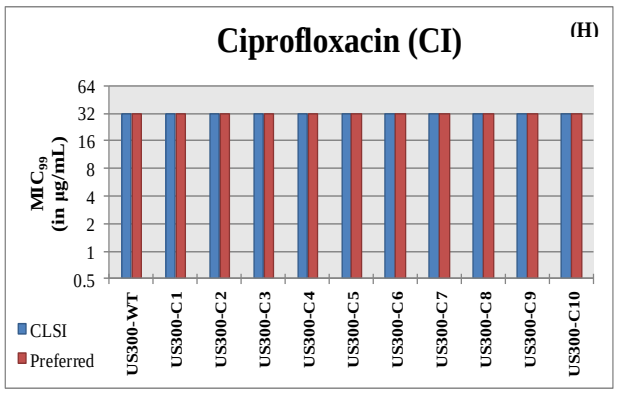



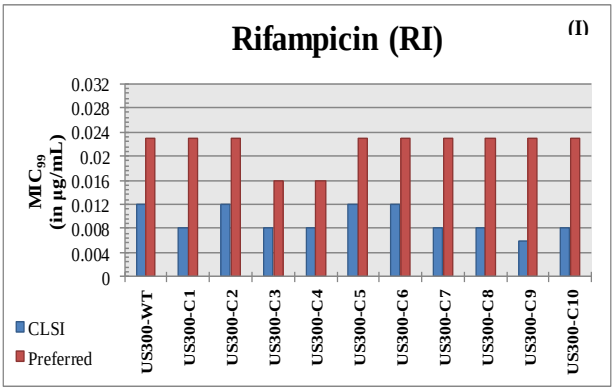



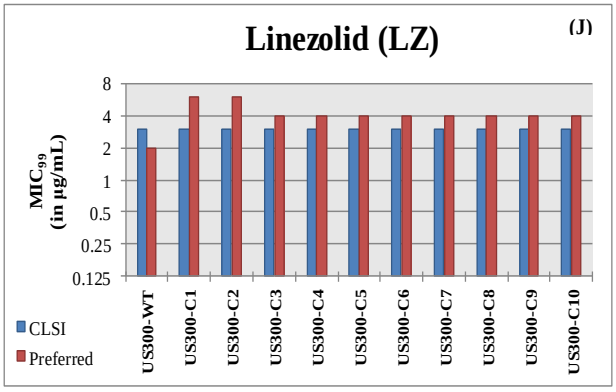



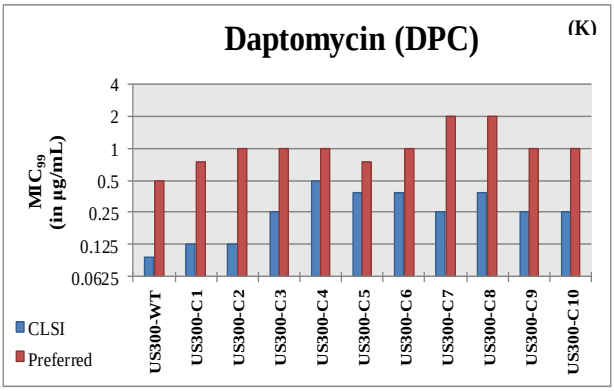






E-tests for 11 antibiotics were conducted on wild-type and mutant samples grown under two different conditions to see the effect of the environment on resistance levels. The extent of resistance appears to vary among the mutant samples, but all mutants possess significantly higher levels of resistance than the wild-type to oxacillin, benzylpenicillin, and cefuroxime. The resistance to CPT, BA, FM, VA, CI, RI, LZ, and DPC seems fairly similar between US300-WT and the mutants. In general, resistance levels appear to be higher under preferred growth conditions, as best evidenced in Figures 5I and 5K, for resistance to rifampicin and daptomycin.

| MID | Reference Position | Type | Reference Base | Called Base | Impact | Feature Type | Feature Name | Amino Acid Change |
|---|---|---|---|---|---|---|---|---|
| US300-C1 | 1775668 | SNP | A | T | Non-synonymous | CDS | clpX | V89D |
| US300-C2 | 1775668 | SNP | A | T | Non-synonymous | CDS | clpX | V89D |

**Table 3: Genome-Wide Comparison of US300-C1 and US300-C2 Paired-End Reads to $t_0$A1 Isolate.** This table is the product of two template assemblies, each comparing a $t_0$A1 and a mutant (either US300-C1 or US300-C2) against a GenBank USA300 wild-type genome. All mutations that were present in both the wild-type and a mutant were removed from the table, leaving only mutations that are unique to one of the mutants. The only distinguishable mutation in US300-C1 is a non-synonymous A-T transversion in the *clpX* gene, which is also unique to US300-C2 when compared to the USA300 wild-type and the $t_0$A1 isolate.

# CHAPTER 4

## Discussion

HBLR is proposed to be responsible for the failure of β-lactams in treating MRSA infections in patients since the weakly resistant majority of cells in a culture will be killed, but a highly resistant minority will survive and continue to damage the host. However, in order to label HBLR as a clinically relevant phenomenon, it is necessary to identify examples of heterogeneous resistance in bacteria subjected to *in vivo* conditions. Specifically, it is necessary to reveal the presence of a highly resistant subpopulation in comparison to a less resistant wild-type.

The purpose of conducting a partial oxacillin PAP was to identify any differences in the OX $MIC_{99}$ of the US300-WT and mutant isolates, and segregate any potential . As evidenced by Figure 3, there is a significant difference between the resistance levels of the wild-type and mutant, as US300-WT possesses a maximum $MIC_{99}$ of 1 μg/mL OX whereas the mutants as a whole have a maximum value of 128μg/mL OX. These two data sets indicate that the mutants isolated from the SEV and *in vivo* model are distinct from the wild-type, suggesting they are the highly resistant subpopulation characteristic of HBLR.

One concern that may arise when determining $MIC_{99}$ from colony counts is that some colonies may be tolerators, rather than resistors. While both types of cells possess heritable traits of resistance, resistors can grow in the presence of antibiotic whereas tolerators are only capable of growth after the antibiotic has degraded. Tolerators, much like persistors, will simply remain dormant or halt growth in the presence of antibiotic to survive treatment (Orman and Brynildsen 2013).

Persistors are excluded from consideration in this experiment since they do not have heritable resistance and the PAP plates were inoculated from an overnight culture. Tolerators, however, could theoretically account for some of the colonies on PAP plates after 72 hours, when the antibiotic has degraded. It is possible to rule out tolerators from interfering with the $MIC_{99}$ for each sample since the mutants and wild-type values sharply diverge from each other after 24 hours, well within the half-life of oxacillin at 37°C in media, so these counts can be attributed to the resistors and not the tolerators, which would be incapable of growth. This is especially noteworthy since the wild-type are killed by a concentration below 10 μg/mL OX, whereas the mutants are all resistant to much higher concentrations, which is consistent with the literature for previous cases of HBLR in staphylococci (Finan, Rosato et al. 2002).

To further demonstrate that the mutant cells are resistors and capable of growing when exposed to oxacillin, it was necessary to observe the early growth of individual cells through phase contrast time-lapse microscopy. As seen in Table 3, both US300-WT and US300-C1 grow well in the absence of oxacillin. However, when cultured on 10 μg/mL OX agar, US300-WT shows no signs of growth, whereas US300-C1 will replicate, albeit at a slower rate than in the absence of antibiotic. Since the time-lapse represents the first 8 hours of growth, it is unlikely that antibiotic would have degraded and, as such, the US300-C1 is a true resistor that can effectively replicate when exposed to oxacillin, rather than a tolerator.

Another item that is noticeable from the time-lapse videos is that US300-C1 appears to grow less than US300-WT from 0 to 4 hours on antibiotic free media, suggesting that there may be a fitness cost associated with increased levels of resistance. This is reaffirmed by the doubling time estimates for the USA300-WT and mutants as seen in Figure 4, as the wild-type has an approximate doubling time of 24 minutes whereas the mutants as a whole have a doubling time of 30 minutes. The specific mechanism behind this fitness cost still remains a mystery.

While *mecA* is necessary for high levels of β-lactam resistance, it is not sufficient, meaning that there are other unknown proteins or molecules that are involved in generating a resistant phenotype. In order to identify some of the unique mutations in the SEV mutants that may contribute to these processes, template assemblies were formed for the initial SEV isolate and either US300-C1 or US300-C2 against the USA300 wild-type. As seen in Table 4, the only distinct mutation possessed by US300-C1 is a non-synonymous transversion in the *clpX* gene, which codes for the ATPase, Clpx (Frees, Qazi et al. 2003). US300-C2 possesses this same *clpX* transversion. ClpX is an important protein in staphylococci that binds to the ClpP subunits of the Clp proteolytic complex, enabling a cell to degrade and recycle any foreign or waste proteins. Normally, loss of function in ClpX will significantly reduce virulence and biofilm formation in *S. aureus*, but there has been no study demonstrating that ClpX mutations can increase resistance (Frees, Chastanet et al. 2004). One theory states that, without functional ClpX, the more essential ClpA ATPase will have greater access to the ClpP subunits and more efficiently recycle proteins in a cell, resulting in greater growth, though this has yet to be proven (Frees, Qazi et al. 2003). Since the same mutation is evident in two of the mutants but absent in the wild-type, it appears that ClpX has some role in the generation of the resistant mutants for HBLR, but this must be better investigated and elaborated upon for confirmation.

Although the partial oxacillin PAP did reveal the OX $MIC_{99}$ for the wild-type and each of the mutants, one goal of the project was to determine whether exposure to oxacillin led to the development of cross-resistance to additional β-lactams or other classes of antibiotics. As seen in Figures 5A-5C, in comparison to US300-WT the mutants have significantly higher levels of resistance under both CLSI and preferred growth conditions to oxacillin, benzylpenicillin, and cefuroxime, all three of which are β-lactams (CLSI 2013). Since all three drugs target PBP2 in order to interfere with proper cell wall synthesis, it stands to reason that resistance to one β-lactam would enable increased resistance to another. β-lactams can, though, still be engineered to

effectively work against the mutants, as evidenced by ceftaroline, a cephalosporin, as seen in Figure 5D (Bazan, Martin et al. 2011). Both US300-WT and the mutants possess relatively equal levels of resistance to ceftaroline, indicating that some β-lactams are still theoretically suitable for treatment of MRSA infections.

Apart from the β-lactams, there is no significant difference between the resistance levels of the wild-type and mutant samples for a given antibiotic, indicating that cross-resistance has not yet developed in the strain.

. This is most likely since few of the remaining seven antibiotics target the cell wall, and some even act through other means to lyse bacteria. Some antibiotics inhibit necessary enzymes in *S. aureus*, such as rifampicin and ciprofloxacin which respectively inhibit RNA polymerase and topoisomerase within a cell, leading to death (Chambers 1997). Antibiotics such as daptomycin, on the other hand, target the outside of the cell, but act on something apart from the cell wall. Daptomycin punctures the cell membrane, ultimately causing depolarization and cell lysis (Rose, Rybak et al. 2007).

Although the main focus of the E-tests were to determine any differences in terms of resistance between the wild-type and mutants, it appears that there is also variation in a sample's resistance level based on its environment. In the case of vancomycin, rifampicin, and daptomycin, as seen in Figures 5G, 5I, and 5K, the inoculated plates under preferred growth conditions possessed higher levels of resistance than similar plates under CLSI conditions. Out of the eleven antibiotics tested in this experiment, only benzylpenicillin and fosfomycin (in Figures 5B and 5F) are less effective under CLSI rather than preferred growth conditions. While the environment does appear to have an impact on the extent of resistance, it appears that the exact temperature and other stresses (such as 2% NaCl in CLSI growth) may confer resistance to one antibiotic over another.

The results of this experiment support the idea that HBLR in USA300 is a clinically relevant phenomenon, as an *in vivo* simulation of the standard treatment for endocardial infection has produced a multitude of bacterial isolates that are much more resistant and otherwise physiologically distinct from the wild-type. With this newfound knowledge in mind, there may be a greater push to develop different treatments methods for USA300 and other similar MRSA infections. While resistance to newer antibiotics such as linezolid is rare, there is a concern that relying on a single antibiotic will simply continue selecting for the subset of highly resistant mutants in a given bacterial population (Gao, Chua et al. 2010). As such, a combinatorial approach to treating MRSA infections may be the most viable course of action. This has already proven successful in the treatment of vancomycin-resistant bacteria, as merely treating a culture with an alternative drug such as rifampicin leads to the emergence of mutants resistant to both drugs. However, when the same culture is exposed to both rifampicin and fusidic acid, no such mutants arise (O'Neill, Cove et al. 2001). While this experiment does support the clinical relevance of HBLR, it is necessary to perform similar studies on additional strains of CA-MRSA to fully confirm the results.

## APPENDIX A

## Abbreviations

**ATCC:** American Type Culture Collection

**BA:** Bacitracin

**CA-US300:** Community-Associated USA300

**CI:** Ciprofloxacin

**CLSI:** Clinical and Laboratory Standards Institute

**CPT:** Ceftaroline

**DPC:** Daptomycin

**FM:** Fosfomycin

**HBLR:** Heterogeneous β-Lactam Resistance

**Indel:** Insertion-deletion mutation

**LZ:** Linezolid

**MHA:** Mueller-Hinton Agar

**MHB:** Mueller-Hinton Broth

**MIC:** Minimum Inhibitory Concentration

**ME:** Methicillin

**MRSA:** Methicillin-Resistant *Staphylococcus aureus*

**OD:** Optical Density

**OX:** Oxacillin

**PAP:** Population Analysis Profile

**PG:** Benzylpenicillin

**PK/PD SEV:** Pharmodynamic/Pharmokinetic Simulated Endocardial Vegetation

**RI:** Rifampicin

**SEV:** Simulated Endocardial Vegetation

**SNP:** Single nucleotide polymorphism

**TSA:** Tryptic Soy Agar

**TSB:** Tryptic Soy Broth

**VA:** Vancomycin

**XM:** Cefuroxime

**WT:** Wild-type

## APPENDIX B

### Additional Data Sets

Each of the following tables (Tables 4A-4K) represents the CFU counts from a single instance of a PAP, where all plates in the profile were inoculated from the same, unique overnight culture of USA300 wild-type or mutant.

**Table 4A:** CFU Counts over Time for US300-WT in Figure 3

| **Plate Antibiotic Concentration (µg/mL OX)** | | **0** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 µL of a $10^{-6}$ dilution of US300-WT on TSA | **t = 12** | 208 | 309 | 218 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 219 | 330 | 225 | 272 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 219 | 330 | 225 | 272 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 48** | 219 | 330 | 225 | 275 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 60** | 219 | 330 | 225 | 278 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 72** | 219 | 330 | 225 | 278 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4B:** CFU Counts over Time for US300-C1 in Figure 3

| **Plate Antibiotic Concentration (µg/mL OX)** | | **0** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 µL of a $10^{-6}$ dilution of US300-C1 on TSA | **t = 12** | 404 | 632 | 540 | 586 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 412 | 644 | 556 | 606 | 598 | 594 | 450 | 500 | 304 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 416 | 644 | 556 | 606 | 598 | 594 | 454 | 522 | 348 | 80 | 0 | 0 | 0 | 0 |
| | **t = 48** | 416 | 644 | 556 | 606 | 598 | 594 | 454 | 522 | 354 | 106 | 0 | 0 | 0 | 0 |
| | **t = 60** | 416 | 644 | 556 | 606 | 598 | 594 | 454 | 522 | 354 | 113 | 0 | 0 | 0 | 0 |
| | **t = 72** | 416 | 644 | 560 | 606 | 598 | 594 | 454 | 522 | 354 | 114 | 0 | 0 | 0 | 0 |

**Table 4C:** CFU Counts over Time for US300-C2 in Figure 3

| **Plate Antibiotic Concentration (µg/mL OX)** | | **0** | **0.125** | **0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** | **64** | **128** | **256** | **512** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 µL of a $10^{-6}$ dilution of US300-C2 on TSA | **t = 12** | 330 | 478 | 394 | 416 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 344 | 482 | 394 | 428 | 492 | 382 | 468 | 302 | 202 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 344 | 482 | 398 | 436 | 492 | 392 | 470 | 310 | 240 | 49 | 0 | 0 | 0 | 0 |
| | **t = 48** | 344 | 482 | 398 | 436 | 492 | 392 | 470 | 310 | 244 | 74 | 0 | 0 | 0 | 0 |
| | **t = 60** | 344 | 482 | 398 | 436 | 492 | 392 | 470 | 310 | 245 | 80 | 0 | 0 | 0 | 0 |
| | **t = 72** | 344 | 482 | 398 | 436 | 492 | 392 | 470 | 310 | 245 | 82 | 0 | 0 | 0 | 0 |

**Table 4D:** CFU Counts over Time for US300-C3 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C3 on TSA | **t = 12** | 388 | 412 | 496 | 538 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 388 | 496 | 520 | 562 | 334 | 474 | 440 | 388 | 236 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 388 | 496 | 524 | 574 | 334 | 478 | 442 | 396 | 274 | 30 | 0 | 0 | 0 | 0 |
| | **t = 48** | 388 | 496 | 524 | 578 | 334 | 478 | 442 | 396 | 274 | 59 | 0 | 0 | 0 | 0 |
| | **t = 60** | 388 | 496 | 524 | 578 | 334 | 478 | 442 | 396 | 274 | 59 | 0 | 0 | 0 | 0 |
| | **t = 72** | 388 | 496 | 528 | 578 | 334 | 478 | 442 | 396 | 274 | 61 | 0 | 0 | 0 | 0 |

**Table 4E:** CFU Counts over Time for US300-C4 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C4 on TSA | **t = 12** | 376 | 516 | 452 | 456 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 396 | 540 | 476 | 484 | 496 | 392 | 376 | 335 | 190 | 1 | 0 | 0 | 0 | 0 |
| | **t = 36** | 396 | 540 | 476 | 484 | 508 | 402 | 384 | 339 | 226 | 48 | 0 | 0 | 0 | 0 |
| | **t = 48** | 396 | 540 | 476 | 484 | 508 | 402 | 384 | 339 | 230 | 66 | 0 | 0 | 0 | 0 |
| | **t = 60** | 396 | 540 | 476 | 484 | 508 | 402 | 384 | 339 | 230 | 68 | 0 | 0 | 0 | 0 |
| | **t = 72** | 396 | 540 | 476 | 498 | 512 | 402 | 384 | 343 | 230 | 68 | 0 | 0 | 0 | 0 |

**Table 4F:** CFU Counts over Time for US300-C5 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C5 on TSA | **t = 12** | 262 | 216 | 296 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 262 | 216 | 296 | 358 | 452 | 272 | 310 | 252 | 85 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 270 | 218 | 300 | 382 | 452 | 272 | 316 | 255 | 110 | 22 | 0 | 0 | 0 | 0 |
| | **t = 48** | 274 | 218 | 304 | 382 | 452 | 272 | 316 | 256 | 112 | 35 | 0 | 0 | 0 | 0 |
| | **t = 60** | 274 | 218 | 304 | 382 | 452 | 272 | 316 | 256 | 112 | 36 | 0 | 0 | 0 | 0 |
| | **t = 72** | 274 | 218 | 304 | 382 | 452 | 272 | 316 | 256 | 112 | 36 | 0 | 0 | 0 | 0 |

**Table 4G:** CFU Counts over Time for US300-C6 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C6 on TSA | **t = 12** | 428 | 498 | 392 | 368 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 428 | 498 | 434 | 432 | 442 | 424 | 580 | 390 | 133 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 448 | 518 | 442 | 432 | 442 | 424 | 582 | 394 | 187 | 45 | 0 | 0 | 0 | 0 |
| | **t = 48** | 448 | 518 | 442 | 440 | 442 | 424 | 586 | 396 | 193 | 58 | 0 | 0 | 0 | 0 |
| | **t = 60** | 448 | 518 | 442 | 440 | 442 | 424 | 586 | 396 | 193 | 61 | 0 | 0 | 0 | 0 |
| | **t = 72** | 448 | 518 | 442 | 440 | 442 | 424 | 586 | 396 | 193 | 61 | 0 | 0 | 0 | 0 |

**Table 4H:** CFU Counts over Time for US300-C7 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C7 on TSA | **t = 12** | 726 | 480 | 428 | 444 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 758 | 484 | 428 | 630 | 586 | 317 | 544 | 300 | 199 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 764 | 484 | 428 | 630 | 586 | 319 | 546 | 305 | 253 | 62 | 0 | 0 | 0 | 0 |
| | **t = 48** | 764 | 488 | 428 | 630 | 586 | 319 | 546 | 305 | 258 | 89 | 0 | 0 | 0 | 0 |
| | **t = 60** | 764 | 488 | 428 | 630 | 586 | 319 | 546 | 305 | 259 | 91 | 0 | 0 | 0 | 0 |
| | **t = 72** | 764 | 488 | 428 | 630 | 586 | 319 | 546 | 305 | 259 | 91 | 0 | 0 | 0 | 0 |

**Table 4I:** CFU Number over Time for US300-C8 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C8 on TSA | **t = 12** | 152 | 135 | 157 | 112 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 158 | 135 | 157 | 114 | 172 | 134 | 125 | 89 | 62 | 0 | 0 | 0 | 0 | 0 |
| | **t = 36** | 159 | 136 | 158 | 114 | 173 | 134 | 125 | 93 | 81 | 7 | 0 | 0 | 0 | 0 |
| | **t = 48** | 159 | 136 | 158 | 114 | 173 | 134 | 126 | 93 | 81 | 8 | 0 | 0 | 0 | 0 |
| | **t = 60** | 159 | 136 | 158 | 114 | 173 | 134 | 126 | 93 | 81 | 13 | 0 | 0 | 0 | 0 |
| | **t = 72** | 159 | 136 | 158 | 114 | 173 | 134 | 126 | 93 | 81 | 13 | 0 | 0 | 0 | 0 |

**Table 4J:** CFU Counts over Time for US300-C9 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C9 on TSA | **t = 12** | 436 | 482 | 368 | 688 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 562 | 538 | 403 | 688 | 526 | 510 | 434 | 343 | 354 | 176 | 0 | 0 | 0 | 0 |
| | **t = 36** | 568 | 574 | 403 | 694 | 526 | 510 | 443 | 344 | 371 | 233 | 2 | 0 | 0 | 0 |
| | **t = 48** | 568 | 574 | 403 | 704 | 528 | 510 | 443 | 345 | 373 | 240 | 11 | 0 | 0 | 0 |
| | **t = 60** | 568 | 574 | 403 | 704 | 528 | 510 | 443 | 345 | 373 | 241 | 17 | 0 | 0 | 0 |
| | **t = 72** | 568 | 574 | 403 | 704 | 528 | 510 | 443 | 345 | 373 | 241 | 18 | 0 | 0 | 0 |

**Table 4K:** CFU Counts over Time for US300-C10 in Figure 3

| Plate Antibiotic Concentration (μg/mL OX) | | 0 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of viable colonies at time *t* (in hours) after plating 50 μL of a $10^{-6}$ dilution of US300-C10 on TSA | **t = 12** | 422 | 536 | 596 | 464 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **t = 24** | 429 | 546 | 616 | 464 | 602 | 652 | 398 | 392 | 376 | 68 | 0 | 0 | 0 | 0 |
| | **t = 36** | 429 | 546 | 616 | 464 | 626 | 662 | 404 | 392 | 380 | 161 | 1 | 0 | 0 | 0 |
| | **t = 48** | 431 | 546 | 616 | 464 | 626 | 662 | 404 | 392 | 382 | 167 | 23 | 0 | 0 | 0 |
| | **t = 60** | 431 | 546 | 616 | 464 | 626 | 662 | 404 | 392 | 382 | 167 | 31 | 0 | 0 | 0 |
| | **t = 72** | 431 | 546 | 616 | 464 | 626 | 662 | 404 | 392 | 382 | 167 | 31 | 0 | 0 | 0 |

**Table 5A:** E-Test $MIC_{99}$ for USA300 WT and Mutant Isolates under CLSI Growth Conditions

| Bacterial Isolate | | US300-WT | US300-C1 | US300-C2 | US300-C3 | US300-C4 | US300-C5 | US300-C6 | US300-C7 | US300-C8 | US300-C9 | US300-C10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $MIC_{99}$ (in µg/mL) | **OX** | 0.5-1 | 64 | 64 | 64 | 96 | 32 | 64 | 64 | 48 | 48 | 64 |
| | **PG** | 0.19-0.25 | 4 | 4 | 6 | 8 | 8 | 8 | 6 | 8 | 6 | 6 |
| | **XM** | 4 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 |
| | **CPT** | 0.38 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| | **BA** | 32 | 48 | 48 | 32 | 32 | 48 | 48 | 32 | 32 | 32 | 48 |
| | **FM** | 1 | 1.5 | 1.5 | 2 | 2 | 2 | 1.5 | 2 | 2 | 2 | 2 |
| | **VA** | 1-1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| | **CI** | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ |
| | **RI** | 0.012 | 0.008 | 0.012 | 0.008 | 0.008 | 0.012 | 0.012 | 0.008 | 0.008 | 0.006 | 0.008 |
| | **LZ** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | **DPC** | 0.094 | 0.125 | 0.125 | 0.25 | 0.5 | 0.38 | 0.38 | 0.25 | 0.38 | 0.25 | 0.25 |

**Table 5B:** E-Test $MIC_{99}$ for USA300 WT and Mutant Isolates under Preferred Growth Conditions

| Bacterial Isolate | US300-WT | US300-C1 | US300-C2 | US300-C3 | US300-C4 | US300-C5 | US300-C6 | US300-C7 | US300-C8 | US300-C9 | US300-C10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $MIC_{99}$ (in µg/mL) | **OX** | 0.5 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 | 64 |
| | **PG** | 0.094 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 4 | 3 |
| | **XM** | 6 | 256+ | 256+ | 256+ | 256+ | 256+ | 256+ | 256+ | 256+ | 256+ |
| | **CPT** | 0.38 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| | **BA** | 32-48 | 64 | 64 | 64 | 48 | 64 | 64 | 32 | 32 | 32 |
| | **FM** | 8 | 128 | 128 | 96 | 96 | 96 | 128 | 96 | 96 | 128 |
| | **VA** | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | **CI** | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ | 32+ |
| | **RI** | 0.023 | 0.023 | 0.023 | 0.016 | 0.016 | 0.023 | 0.023 | 0.023 | 0.023 | 0.023 |
| | **LZ** | 2 | 6 | 6 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | **DPC** | 0.5 | 0.75 | 1 | 1 | 1 | 0.75 | 1 | 2 | 2 | 1 |

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# ACADEMIC VITA

# GEORGE ANDREW S. INGLIS

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## EDUCATION

FALL 2010 – PRESENT THE PENNSYLVANIA STATE UNIVERSITY UNIVERSITY PARK, PA

**EBERLY COLLEGE OF SCIENCE & SCHREYER HONORS COLLEGE**

Bachelor of Science in Biology, *Genetics & Developmental Biology Option*
Microbiology Minor

**DEAN'S LIST:** Fall 2010 – Spring 2013

## INDEPENDENT RESEARCH EXPERIENCES

MICHAEL MWANGI GROUP: Research Assistant
*Fall 2012 – Present*

- Conduct honors thesis research relating *in vitro* models of antibiotic resistance in US300 to clinical *in vivo* models. This entails analyzing mutants that have survived a 72-hour oxacillin treatment simulating the conditions of an antibiotic-resistant strain in the human heart during a case of endocarditis.
- Determine the extent of cross-resistance in US300 mutants through additional treatments including E-tests and growth under preferred and CLSI conditions in the presence of antibiotic.
- Characterize US300 mutants through population analysis profiles, doubling time experiments, genomic sequencing, and time-lapse microscopy.

BLAIR HEDGES GROUP: Research Assistant
*Fall 2010 – Winter 2012*

- Examined genetic and morphological variation among populations of individual species of Hispaniolan frogs (particularly in the genus Eleutherodactylus).
- Completed a 10-week research initiative from May to August 2011, examining and cataloguing 14 species of frogs, extracting and purifying mitochondrial DNA, and creating phylogenetic trees to compare sequence data.

## LABORATORY SKILLS & TECHNIQUES

-(Touchdown) PCR
-(g)DNA Extraction & Purification
-BLAST
-Time Lapse Microscopy
-Growth Media & Bacterial Stock Preparation
-Agarose Gel Electrophoresis
-DNA Sequencing & Analysis
-Population Analysis Profiles
-Bacterial Doubling Time Estimation

## ACADEMIC & TEACHING EXPERIENCES

PENNSYLVANIA STATE UNIVERSITY BIOLOGY DEPARTMENT: Teaching Assistant
*BIOL 110H, Honors Basic Concepts & Biodiversity (Fall 2012 & Fall 2013)*
*BIOL 220W, Populations and Communities (Spring 2014)*
*BIOL 110, Basic Concepts & Biodiversity (Spring & Summer 2013)*

- Instructed section of 24-27 students in basic laboratory procedures and practices, including writing reports, maintaining a notebook, and designing an experiment.
- Guided students through two major experiments culminating in formal reports.
- Responsible for developing presentations, quizzes, and educational materials for students on a weekly basis in addition to grading assignments.

BIOLOGY 322, GENETIC ANALYSIS: Teaching Assistant
*Spring 2013*

- Led weekly problem-solving sessions concerning intermediate topics in genetics, including polygenetic inheritance, gene regulation, and linkage mapping.

HONORS EDUCATION AT RESEARCH UNIVERSITIES CONFERENCE: Presenter
*Summer 2013*

- Presented to representatives from over 20 universities and colleges at the inaugural Honors Education at Research Universities (HERU) Conference, titled "Successful Programming Model for First-Year Orientation."
- Elaborated on methods of creating beneficial orientation programs for incoming honors students, and the value of such programs.

## LEADERSHIP EXPERIENCES

SCHREYER HONORS COLLEGE ORIENTATION: Lead Mentor
*Fall 2012 – Summer 2013*

- Coordinated with both University faculty and over a hundred other honors students to develop and execute a three day orientation (SHO TIME) for the Schreyer Honors College Class of 2017.

THE PENN STATE THESPIANS: Vice-President
*Summer 2013 – Spring 2014*

- Serve as parliamentarian for the University's oldest continuously run student organization, ensuring that the constitution is in accordance with University policies.
- Actively recruit prospective Thespians and guide them through the two-semester process to become an active member.

NONE OF THE ABOVE: President & Business Manager
*Fall 2011 – Spring 2013*

- Arranged performances, booked concerts and rehearsal spaces, and managed all of the business and logistical operations of the University's oldest co-ed a cappella group.