TRANSFERENCE OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* USING ANTIBIOTIC CHALLENGE

By

TRANG XUAN PHAM

Bachelor of Science in Biochemistry

Wichita State University

Wichita, KS

2008

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2011

TRANSFERENCE OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* USING ANTIBIOTIC CHALLENGE

Thesis Approved:

Dr. Tom Glass
Thesis Adviser

Dr. Robert Allen
Committee Member

Dr. Stanley Conrad
Committee Member

Dr. Gerwald Köhler
Committee Member

Dr. Sheryl A. Tucker
Dean of the Graduate College

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

ABI - Applied Biosystems

ATCC - American Type Culture Collection

BAP - Blood agar plate

BHI - Brain Heart Infusion

CFU - Colony forming units

CLSI - Clinical Laboratory Standards Institute

DNA - Deoxyribonucleic Acid

EDTA - Ethylendiaminetetraacetic acid

gDNA - Genomic deoxyribonucleic acid

MRSA – Methicillin resistant *Staphylococcus aureus*

PBP – Penicillin-binding protein

MHA – Mueller Hinton agar

MHB - Mueller Hinton broth

MOD- PBP – Modified penicillin-binding protein

MIC- Minimum Inhibitory Concentration

MSSA - Methicillin sensitive *Staphylococcus aureus*

NaCl - Sodium chloride

PCR – Polymerase Chain Reaction

ProK- Proteinase K

RFU – Relative fluorescence units

SCC*mec*- Staphylococccal Cassette Chromosome *mec*

SDS- Sodium dodecyl sulfate

TE – Tris + EDTA

TNE – Tris+ NaCl (sodium chloride)+ EDTA

UV - Ultraviolet

Chapter I. Introduction

The increasing incidence of methicillin resistance in *Staphylococcus* infections has created a therapeutic challenge that necessitates a better understanding of antibiotic resistance (Arias & Murray, 2009). Methicillin-resistant *S. aureus* (MRSA) tends also to be resistant to many other antibiotics currently used in the treatment of infections. By understanding how methicillin resistance is inherited and maintained by *S. aureus*, the spread of MRSA could potentially be prevented by appropriate therapeutic measures.

S. aureus is a gram-positive spherical bacterium, commonly found in the nares and on the skin of 25 to 30% of the U.S. population (CDC, 2011). *S. aureus* causes both local and systemic diseases; including soft tissue infections, scalded skin syndrome, toxic shock syndrome, osteomyelitis, and pneumonia (Goetghebeur, Landry, Han, & Vincente, 2006). Staphylococcal infections can be particularly harmful to immunocompromised individuals.

Recent research has shown that antimicrobial therapy may be a major factor associated with the development of methicillin resistance. Treatment with multiple drugs may result in multiple resistances, thereby increasing the difficulty for successful treatment (Lu, et al, 2005). Understanding these relationships is essential in the detection and treatment of MRSA.

A major factor in *S. aureus* methicillin resistance is the *mecA* gene complex. This

gene has been identified in MRSA isolates and is absent in methicillin-susceptible *S. aureus* (MSSA) (Petersson, Kamme, & Miorner, 1999). The *mecA* gene is located on a 21-kb to 67-kb mobile element known as the staphylococcal cassette chromosome *mec* (SCC*mec*). The mobility of this element contributes to its reputation as the major component of gene transference (Wielders, Fluit, Brisse, Verhoef, & Schmitz, 2002). The *mecA* gene product is responsible for the general ineffectiveness of ß-lactam antibiotics, including methicillin. *mecA* expression results in the production of penicillin-binding protein 2a (PBP 2a). Beta-lactam antibiotics such as methicillin inhibit bacterial cell wall synthesis by binding to normally occurring penicillin-binding proteins (PBP). Insensitivity to methicillin is due to the low binding affinity of *mecA*-encoded PBP 2a, a transglycosylase and transpeptidase molecule (Walsh, 2003). Therefore, the resistance of *S. aureus* to methicillin and other ß -lactam antibiotics can be determined by the expression of the *mecA* gene or the presence of the PBP 2a protein. However, it must be noted that methicillin resistance cannot be solely attributed to *mecA* expression as other factors may be involved. Intrinsically produced modified penicillin-binding proteins (MOD-PBPs) and overproduction of *β*-lactamases are also considered mechanisms of resistance (Tomasz et al., 1989, McDougal & Thornsberry, 1986).

The origin of *mecA* in *S. aureus* has not been elucidated, however; many hypotheses support the idea of interspecies transfer. Epidemiology suggests that acquisition of *mecA* is accomplished through horizontal gene transfer. Genetic material can be transferred between bacteria by three known mechanisms: transduction, conjugation, and transformation. Transduction is the transfer of bacterial genetic material from one bacterium to another via a bacteriophage. Conjugation requires cell-to-cell

contact and transfer of genetic material incorporated into a plasmid via a pilus. Transformation occurs when a bacterial cell takes up foreign DNA from the environment and incorporates it into its genome for subsequent expression. This study focuses on transformation as a potential mechanism for *mecA* transference. The abundance of DNA in the environment resulting from cellular lysis provides a rich pool of genetic material available to bacteria. The aim of this research is to document the transference of a functional *mecA* gene from MRSA genomic DNA to methicillin-sensitive *S. aureus* thereby converting the MSSA to a MRSA.

Chapter II. Review of Literature

2.1. *Staphylococcus aureus* Infections

2.1.1. Pathogenicity of MRSA

Staphylococcus species are ubiquitous in nature. The CDC estimates that 25 to 30 percent of both healthy individuals and immunocompromised patients harbor this microorganism (CDC, 2011). Diseases caused by *S. aureus* range from localized skin rashes to life-threatening systemic diseases. Pathogenicity of *S. aureus* is associated with a number of virulence factors. Protein A is an example of a surface factor that allows the bacterium to evade phagocytosis by binding to IgM. Other virulence factors contribute to disease by interfering with the body's immune response. Degradative enzymes such as hyaluronidase, nuclease, and protease are examples of virulence factors that facilitate the spread of the bacterium. *S. aureus* can attack the immune system directly by secretion of hemolysins and leukocidins; toxins that are capable of disabling and killing phagocytes. Pyrogenic toxins act as superantigens, causing a harmful release of cytokines. Staphylococcal enterotoxins can cause vomiting, diarrhea, and related symptoms of food poisoning. Toxic Shock Syndrome (TSS) is commonly associated with *S. aureus* and is a result of the secretion of the Toxic Shock Syndrome Toxin (TSST-1). Symptoms associated with this syndrome include a superantigen response of inflammation, fever,

hypotension, and potentially septic shock. Additionally, staphylococcal scalded skin syndrome (SSSS) causes severe blistering of the skin due to exfoliative toxins produced by the microorganism (Wing & Kulkarni, 2010). The relationship between these virulence factors and MRSA-related diseases has not been clearly established. The cumulative effects of pathogenicity and methicillin resistance are self-evident.

2.1.2. Morbidity/Mortality

S. aureus may colonize the nasal passages and/or skin of asymptomatic carriers. MRSA infections can result in sepsis, cellulitis, endocarditis, pneumonia, and/or TSS. The cumulative effects of these infections are highly dependent on the overall health of the individual. A weakened immune system coupled with a serious staphylococcal infection may be lethal as demonstrated by the high mortality rates associated with MRSA-related pneumonia and sepsis (Dugdale & Vyas, 2009).

2.2. Antibiotic Treatments

2.2.1. Methicillin and Other Structurally-related Compounds (Oxacillin)

Resistance to penicillin resulted in the use of methicillin as an alternative treatment for *S. aureus* infections (Enright *et al*., 2002). A few years after the introduction of methicillin, methicillin resistance was detected in the United Kingdom (1961). As a beta-lactam antibiotic, methicillin is structurally related to penicillin and cephalosporin. The binding of methicillin to intrinsically produced penicillin-binding

proteins (PBPs) inhibits cell wall synthesis. PBP acts as a catalyst in the cross-linking of peptides in the peptidoglycan component of the staphylococcal cell wall. Interference with bacterial cell wall synthesis is disruptive to the normal maintenance and growth of bacteria, resulting in cellular death. Methicillin is no longer commercially produced in the United States and has been replaced by oxacillin in clinical use (CDC, 2011). Although oxacillin is used clinically to test for resistance, resistant strains are still referred to as methicillin-resistant *S. aureus* due to its historical significance.

2.2.2. Multiple Resistance

Treatment of MRSA infections becomes even more problematic when resistance is not limited to methicillin, but also to other commonly used antibiotics. Clinical research has demonstrated that antibiotic treatment may become a major factor in the colonization of MRSA. Treatment of MRSA infections with multiple antibiotics has contributed to the already difficult process of eliminating the bacteria (Lu, *et al.*, 2005). *S. aureus* that are resistant to methicillin are usually resistant to other beta-lactam antibiotics, including penicillin G and cephalosporins (Katayama, Ito, & Hiramatsu, 2000). The factors contributing to resistance in MRSA are similar to penicillin G resistance in strains of *S. aureus* (Chambers, 2001). MRSA can also exhibit resistance to aminoglycosides and tetracycline (Parker & Hewitt, 1970). MRSA infections are generally treated with a combination of antibiotics including: clindamycin, daptomycin, doxycycline, linezolid, minocycline, tetractycline, trimethoprim-sulfamethoxazole, vancomycin (Dugdale & Vyas, 2009).

2.3. Epidemiology

2.3.1. Classification

Resistance to methicillin has been reported in hospitals as well as in the community. MRSA infections are currently classified as either community-acquired (CA-MRSA) or hospital-acquired MRSA (HA-MRSA). It has been postulated that MRSA originated in the hospital environment due to the indiscriminate use of antibiotics (Chambers, 2001). In contrast, MRSA infections are considered to be community-acquired (CA-MRSA) only if the patient meets certain criteria such as no recent surgery, no implanted medical devices, or no recent antibiotic therapy. In addition, the patient should not have taken residence in a long-term care facility nor have been recently hospitalized with a history of HA-MRSA (Demling & Waterhouse, 2007). Several studies have attempted to identify genetic differences in order to characterize and classify CA-MRSA and HA-MRSA.

2.3.2. Difference between CA-MRSA and HA-MRSA

Strains of MRSA originating from hospitals (HA- MRSA) are generally resistant to multiple antibiotics in contrast to their community-acquired counterpart (CA-MRSA) (Chambers, 2001). This finding can be explained by the lower antibiotic selective pressures in the community as compared to the hospital environment. However, this epidemiology of *S. aureus* has changed substantially in recent years. The dissemination of HA- MRSA into the community has made it difficult to distinguish between HA- and CA-MRSA. The origin of CA-MRSA has been speculated to be the consequence of horizontal gene transfer of *mecA* into formerly susceptible strains (Chamber, 2001).

Molecular typing of CA-MRSA and HA-MRSA has demonstrated differences in their antibiotic susceptibilities. HA-MRSA commonly contain resistance genes to other antibiotics in addition to the methicillin resistance determinant which are generally absent in CA-MRSA. In 2007, Boyle-Vavra and Daum examined the correlation between virulence and MRSA. They identified a possible link between CA-MRSA and Panton-Valentine Leukocidin (PVL) toxin.

2.4. Methicillin Resistance

2.4.1. Resistance Mechanisms

S. aureus is able to circumvent the lethal effects of methicillin by a combination of multiple mechanisms of resistance. One of those mechanisms is the production of modified penicillin-binding proteins (MOD-PBPs). Methicillin acts by binding to intrinsically produced PBPs thereby inhibiting cell wall synthesis. MOD-PBPs have a substantially reduced affinity for methicillin as compared to normal PBPs allowing near normal levels of cell wall synthesis (Tomasz et al., 1989). In addition to MOD-PBPs, a second mechanism of resistance is the overproduction of beta-lactamases that can inactivate methicillin. The large amount of beta-lactamase produced can lead to the rapid hydrolysis of penicillin and partial hydrolysis of penicillinase-resistant antibiotics such as oxacillin, cephalosporins, and methicillin (McDougal & Thornsberry, 1986) thereby conferring resistance. These mechanisms have been more commonly associated with borderline-resistant *S. aureus*. Classical resistance is primarily attributed to the methicillin resistance determinant (*mecA*) which is detectable in MRSA isolates while absent in MSSA (Petersson, Kamme, & Miorner, 1999). This gene codes for penicillin-

binding protein 2a (PBP 2a) (Archer, et al., 1994) and is located on a novel genetic mobile element, Staphylococcal Cassette Chromosome *mec* (SCC*mec*).

2.4.2. Defining Resistance

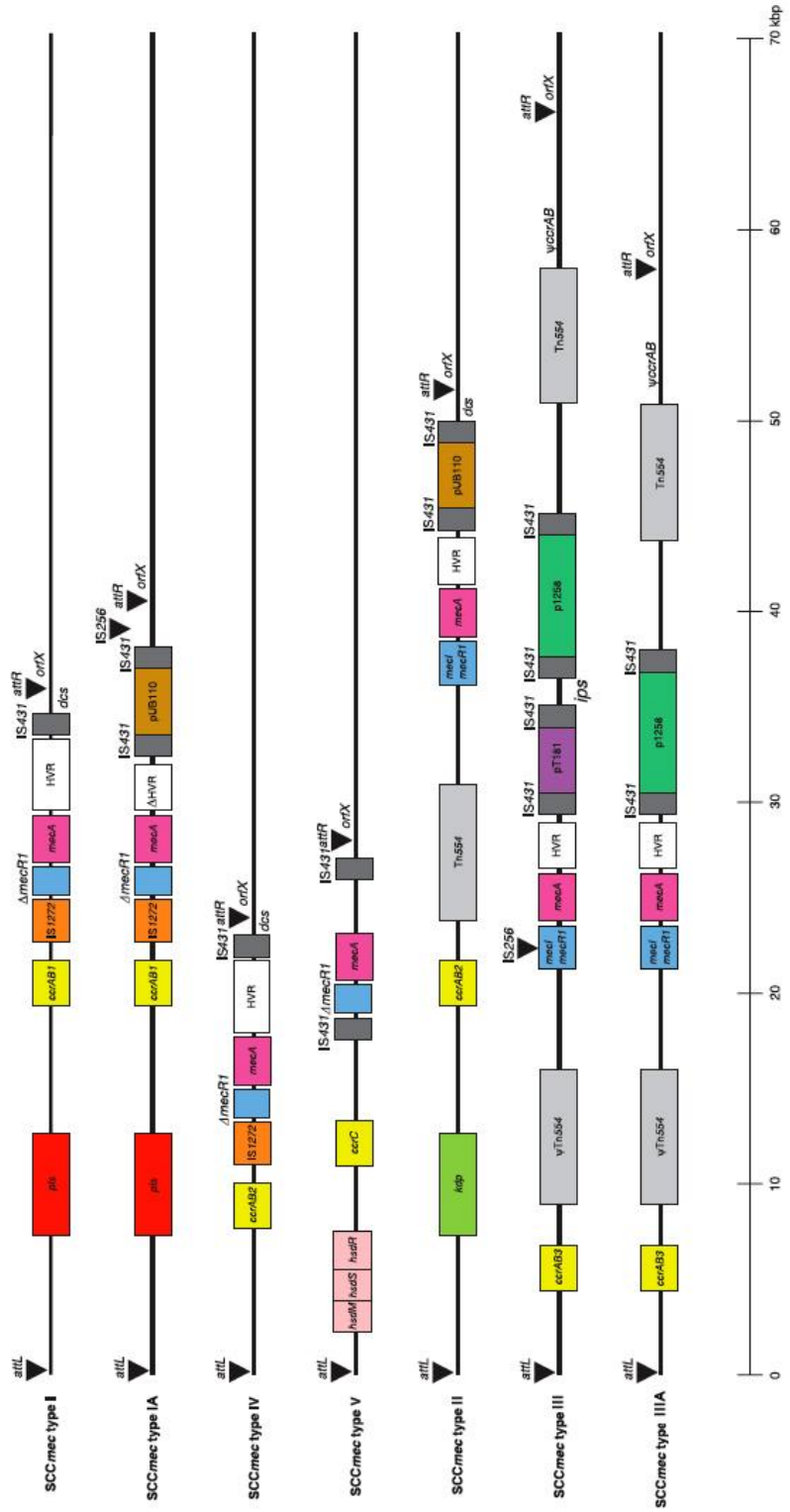
Expression of methicillin resistance (*mecA)* in MRSA is characteristically heterogeneous. This heterogeneity is ill-defined and results in difficulties detecting methicillin resistance. A culture of MRSA may contain two subpopulations; one susceptible and the other resistant to methicillin. Although all of the cells in that culture may be genetic clones containing *mecA*, not all of the cells may express the gene therefore appear to be sensitive to methicillin. The Clinical Laboratory of Standards Institute (CLSI) developed standard protocols for the identification of MRSA and MSSA. They use several methods to phenotypically distinguish MRSA from MSSA. CLSI established the interpretive criteria for MIC oxacillin susceptibility as less than 2 μg/ml for MSSA and greater than 4 μg/ml for MRSA (Murray *et al.*, 2009). The polymerase chain reaction (PCR) is the accepted standard used to confirm the presence of the *mecA* gene.

2.4.3. SCC*mec* Types

Molecular typing of the staphylococcal cassette chromosome *mec* (SCC*mec*) isolated from MRSA has revealed differences in size and sequence of the genetic element. At least five different types of SCC*mec* (I - V) have been identified thus far (Figure 1). The different SCC*mec* types range in size between 21- to 67-kb and differ in their combinations of complexes. The mobile element contains two main complexes: a *mec* complex and a cassette chromosome recombinase (*ccr*) complex. In addition to the

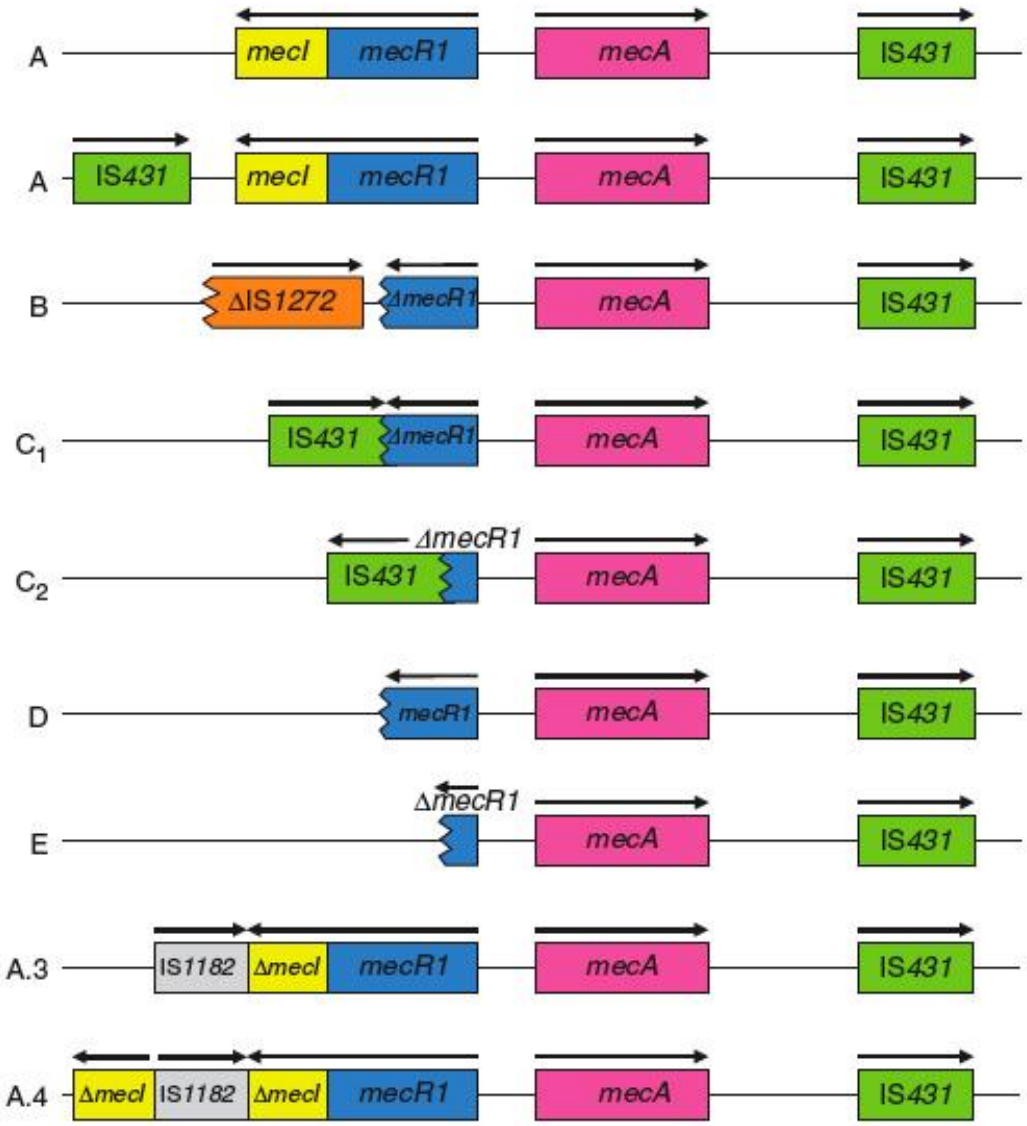
ccr and *mec* complexes, SCC*mec* is comprised of a J-region (Junkyard region), which can also be used to distinguish among various SCC*mec* types (Hanssen & Sollid, 2006). The *mec* complex contains the *mecA* gene and is categorized into different classes with different combinations of *mecI* and *mecR* (Figure 2). Expression of the *mecA* gene is regulated by the *mecR1-mecI*, a two gene operon that is located upstream from *mecA*. The *mecR1* gene encodes a signal sensor and *mecI* encodes a transducer (Ito et al., 1999 & Ito et.al, 2004). There are five different known allotypes of *ccr*. The *ccr* complex codes for site-specific recombinases that effectively, excise and integrate SCC*mec* into the chromosome (Katayama et al., 2000). Therefore, the mobile element in which *mecA* is located can be specifically integrated into the chromosome of *S. aureus* and potentially lead to the expression of methicillin resistance. The mobility of this element gives rise to the possibility of transference of methicillin resistance between bacterial cells. SCC*mec* types I, IV, and V do not contain any antibiotic resistance genes other than the methicillin resistance determinant. They are generally associated with CA-MRSA (Hanssen & Sollid, 2006).

Figure 1. SCC*mec* types (I-V)



*Illus. in Chambers, 2001. Identified SCC*mec* mobile elements containing a combination of complexes (*mec* and *ccr* complexes) and of varying sizes (21- 67 kb)

Figure 2. *mec* gene complexes



* Illus. in Chambers, 2001. The *mec* gene complexes present in different SCC*mec* mobile elements.

2.4.4. Stability of *mecA*

The SCC*mec* element harbors the *mecA* gene complex which codes for penicillin-binding protein 2a, a 76 kDa protein (Tomasz, et al., 1989). As such, PBP 2a production is responsible for methicillin resistance. PBP 2a has a lower binding affinity to methicillin which enables cell wall assembly and subsequently confers resistance. Transfer of this gene may provide a selective advantage for *S. aureus* under antibiotic pressure. However, the acquisition and maintenance of the *mecA* complex requires increased energy demands on the bacteria, not found in non-*mecA* cells and apparently comes at a fitness cost to the bacterial cell. Cells containing the *mecA* complex grow at a slower rate in order to maintain relatively large amounts of SCCmec (Ender, et al., 2004). A longevity study conducted by Griethuysen *et al* (2005) demonstrated that MRSA strains can lose the *mecA* gene after years of storage at -80°C. Chambers (2001) stated "only a handful of ancestral strains account for all clinical isolates worldwide." The acquisition of *mecA* may therefore only prove to be a transient advantage under selective conditions.

2.5. Transference

2.5.1. Mechanisms of Genetic Exchange

Although *mecA* is widely distributed among *S. aureus*, its origin is still obscure. Epidemiological studies have suggested that methicillin resistance in *S. aureus* is acquired via horizontal gene transfer. The transfer of genetic information can occur by at least three known mechanisms: transduction, conjugation, and transformation.

Transduction is the process by which a bacteriophage mediates DNA transfer from the donor cell to the recipient cell. Conjugation is a process whereby DNA transference is medicated by plasmids. Plasmids are extrachromosomal genetic elements that may contain resistance genes. Conjugation requires cell-to-cell contact, generally via pili to transfer plasmids from the donor cells to the recipient cells (McClane, et al., 1999). Transformation occurs when the recipient cell incorporates naked DNA that is released from the donor cells. DNA from a lysed donor cell can be incorporated into a recipient cell and integrated into its genome. The term "natural transformation" refers to the uptake of DNA by naturally competent cells (Chen & Dubnau, 2004). Limiting factors in the uptake of DNA by competent microorganisms may include: the organism's ability to take up, transport, and incorporate the DNA. This condition is described by Hanssen & Sollid "There is a pool of virulence- and antibiotic- resistance genes in the environment in the form of large elements available for transfer between strains." (Hanssen & Sollid, 2006).

2.5.2. Gene Transfer in Staphylococci

Penicillin resistance is more readily transferred when located on plasmids than when incorporated into the cellular DNA (Chambers, 2004). The methicillin resistance determinant on SCC*mec*; however, is chromosomally encoded. The transference of *mecA* harboring SCC*mec* is ill-defined. *In vivo* interspecies transfer of *mecA* has been observed (Wielders, et al., 2001). In this study, a methicillin-sensitive strain of *S. aureus* acquired *mecA* while in the presence of *mecA*-positive *S. epidermidis* and antibiotic treatment. Prior to antibiotic treatment, no *mecA*-positive *S. aureus* were isolated from the patient in the study. Horizontal gene transfer was suggested because the *mecA*-negative *S. aureus*

isolated prior to antibiotic treatment was isogenic to the *mecA*-positive *S. aureus* isolated after antibiotic exposure. This study demonstrates the possibility of *mecA* transference to methicillin-sensitive *S. aureus.*

Chapter III. Methods

3.1. Bacterial Isolates

3.1.1. Staphylococcal strains

Staphylococcal strains used in this study included 10 *S. aureus* strains previously isolated from mouth-guards and band musical instrument studies (Glass, *et al*., 2009 & Glass, *et al*., 2011) and 18 de-identified MRSA isolates from St. John's hospital, Tulsa, OK. Additionally, three staphylococcal reference strains ATCC 27626, ATCC 29213, and ATCC 25923 (Washington D.C., ATCC) were used as controls.

3.1.2. Characterization

Identity of all strains was confirmed using standard procedures including, Gram stains, coagulase, and mannitol salt agar reactions. Ten strains of confirmed *S. aureus* were further characterized as phenotypically MRSA or MSSA by standard antibiotic sensitivity.

3.1.3. Storage

Stock cultures were maintained in Brain Heart Infusion (BHI) (Accumedia, Calgary, Canada) containing 20% glycerol and were stored at -80°C in cryogenic vials until used.

3.2. Screening

Oxacillin resistance levels of bacterial strains were measured by standard macro-broth dilution methods. This method allows for the determination of the minimum inhibitory concentration (MIC) of oxacillin on *S. aureus* isolates. The MIC level indicates the lowest concentration of antibiotic that inhibits the visible growth of a microorganism.

3.2.1. Media

The broth macro-dilution method was employed using Mueller-Hinton Broth (MHB) (Sparks, MD, Difco) supplemented with 2% NaCl (Mallinkrodt, Paris, KY) as recommended by the CLSI (National Committee for Clinical Laboratory Standards) (Murray, et al.).

3.2.2. Antibiotic Dilutions

A 1 mg/ml oxacillin (St. Louis, MO, Sigma) stock solution was used. Serial dilutions of 128-, 64-, 32-, 16-, 8-, 4-, 2-, and 1-µg/ml oxacillin were prepared in sterile culture tubes containing 5 ml of MHB. Positive and negative controls were used. Neither the positive nor negative control contained any oxacillin. The positive control was inoculated with the test strain whereas the negative control was not. Additionally, for MSSA a lower MIC range had to be established to determine the MIC value. This was established by serial dilutions of 1-, 0.5-, 0.25-, 0.125-, 0.0625-, 0.03125-µg/ml oxacillin, prepared in 5 ml of MHB. Positive and negative controls were also used .

3.2.3. Inoculate

A 0.5 McFarland standard was used as a reference to prepare the initial inoculums (Andrews, 2001). Colonies were grown overnight on Blood Agar Plates (BAP) (BBL, Sparks, MD) at 37°C then suspended in MHB until the broth reached a 0.5 McFarland Standard turbidity (McFarland Standard). After serial dilution of antibiotics, each tube (except the negative control) was inoculated with a concentration of 5×10^5 CFU/ml of the test strain. The tubes were incubated for aeration in an Orbit Environ-Shaker shaker (Lab-line Instruments Inc. Melrose Park, IL) at 37°C for 24 hours. The MIC values of each strain were recorded as the lowest concentration in which no visible growth was observed.

3.3. Genetic Analyses

Genetic analyses of test strains indicated the presence of target genes, specifically the *mecA* gene. Only strains that qualify under the designated phenotypic and genotypic criteria were utilized for the transformation experiments.

3.3.1. DNA Isolation

DNA extraction was initially performed using the Zymo Research Bacterial Fungal Kit (Zymo Research, Irvine, CA). The protocol (Appendix A) was followed with minor modifications. Each bacterial strain was cultured in 10 ml of MHB overnight at 37°C. Cells were transferred to appropriate tubes and centrifuged at 3,000 x g for 5 minutes. Pelleted cells were resuspended in 200 µl of UV-treated sterile water and transferred to *ZR Bashing Bead Lysis Tubes*©. After the addition of 750 µl of *Lysis*

Solution, the tubes were secured in the bead-beater (Mini-Beadbeater-96, BioSpec Products, Bartlesville, OK) and processed at maximum speed for two 3-minute sessions with a 1-minute resting period in between sessions. Following the bead-bashing step, extraction proceeded as stated in the provided ZR kit protocol. A NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE) was used to quantitate DNA concentration and purity of the extracted DNA (260/280 nm).

3.3.1.1. Phenol: Chloroform DNA Extraction

An alternative DNA extraction method, by phenol/chloroform, was employed to isolate intact DNA. Aliquots (1 ml) of overnight cultures were transferred into microcentrifuge tubes and centrifuged at 3000 x g for 5 minutes. Cells were then resuspended in 0.4 ml of TNE containing 2 mg/ml lysozyme and 50 U/ml lysostaphin. Samples were then incubated at 37°C for 1 hour. Following incubation, Proteinase K (ProK) (400 μg/ml) and SDS (0.2%) were added to the samples and incubated at 65°C for 1 hour. Samples were extracted with an equal volume of phenol: chloroform: isoamyl alcohol (9:0.96:0.4) and then centrifuged at 10,000 x g for 3 minutes. The aqueous layer was transferred to a clean tube and extracted with an equal volume of chloroform: isoamyl alcohol (24:1). Samples were then centrifuged again at 10,000 x g for 3 minutes. The aqueous layer was transferred to another clean tube and 2 volumes of 95% ethanol were added to precipitate DNA. Clots of DNA were looped out and resuspended in 100 μl of TE^{-4} buffer (10 mM Tris + 0.1 mM EDTA, pH 8.0). DNA concentration was measured by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) with a purity value between 1.8-1.9 (260/280 nm). After isolation,

amplification and capillary electrophoresis were run as described in sections 3.3.2 and 3.3.3.

3.3.2. Polymerase Chain Reaction (PCR)

3.3.2.1. Primers

Polymerase chain reactions were employed to amplify targeted gene sequences. Specific fluorescent labeled primers (Invitrogen, Carlsbad, CA) were designed for targeted gene sequences including: methicillin resistance gene *mecA*, penicillin resistance gene *blaZ*, coagulase gene *coA*, and housekeeping gene *htrA*. Sequences of the primers and PCR product sizes are listed in **Table 1**.

Table 1. Primer description

Primers		Sequence	Fluorescent Dye	Product Length
blaZ	Forward	5'- TTG CTG ATA AAA GTG GTC AAG -3'	ROX (red)	77
	Reverse	5'- AGA TTG GCC CTT AGG ATA AAC -3'		
coA	Forward	5'- GCT TCT CAA TAT GGT CCG AG -3'	FAM (blue)	132
	Reverse	5'- CTT GTT GAA TCT TGG TCT CGC -3'		
mecA	Forward	5'- TTT TGA TCC ATT TGT TGT TG -3'	ROX (red)	153
	Reverse	5'- AAT TTT AGA CCG AAA CAA TGT G -3'		
htrA	Forward	5'- TAA ATC GGT CGT TAC AGT TGA -3'	ROX (red)	169
	Reverse	5'- TGA TTT TCT TTA TCA CCG ACA -3'		

coA primers sequence retrieved from Jones *et al.*, 1996.

3.3.2.2. Sample Amplification

Previously extracted DNA was used as template DNA for the PCR. Samples were prepared as indicated in Table 2. All primers were ordered from Invitrogen (Carlsbad, CA). The master mix contained 10 X Gold St*r Buffer (Promega Corp, Madison, WI),

AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), template DNA (1 ng/μl), and PCR water. Separate PCR were prepared for each primer pair. Forward and reverse primers were added to the following final concentrations in each sample: *coa* (1X) - 2 μM, *mecA* (3X) – 3 μM, *htrA* (2X)- 2 μM, and *BlaZ* (1X)- 1 μM.

Table 2. PCR volumes

	Volume (μl)/ sample
***coA* (1X), *mecA* (3X), *htrA* (2X), *BlaZ* (1X)**	1.25
Gold St*r Buffer (10X)	1.25
Taq Polymerase	0.25
PCR Water	8.75
Template DNA (1 ng/μl)	1.0
Total Volume	**12.5**

Samples were amplified in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) under the specified parameters listed in Table 3.

Table 3. Thermal cycling parameters for PCR

1 Cycle	25 Cycles			1 Cycle	
Initial Incubation	Denature	Anneal	Extend	Final Extension	Final Step
96°C	94°C	60°C	72°C	72°C	4°C
11 min	30 sec	30 sec	30 sec	1 hour	Hold

3.3.3. Capillary Electrophoresis

Analyses of the PCR products were accomplished using capillary electrophoresis. The ABI Prism Genetic Analyzer 310 (Applied Biosystems), a single injection capillary

electrophoresis system, was used to detect fluorescently tagged amplification products. Samples were prepared by adding 1 µl of PCR product to 25 µl of the loading solution containing Hi-Di formamide/ GeneScan Liz 500 size standard (100:1) (Applied Biosystems). Samples were run under specified parameters: run time = 20 minutes, run temp = 60 C, injection time = 5 seconds.

3.4. Transformation

Prior to the transformation experiments, the phenotypic and genotypic profiles of the potential recipient MSSA strains: HMG-5E3 ("5E3") and HMG-5G8 and donor MRSA strains: SJ #3 and SJ #6 were confirmed. Macro-broth dilutions, DNA isolation, amplification, and capillary electrophoresis were performed as per protocols listed previously in sections 3.2, 3.3.1, 3.3.2.2, and 3.3.3, respectively.

3.4.1. Feasibility Test

The possibility of transforming MSSA strains to MRSA strains was evaluated by feasibility tests. Samples were prepared in sterile borosilicate test tubes (VWR, Radnoor, PA). Strain 5E3 was selected as the recipient MSSA test strain. Genomic DNA previously isolated using the Zymo Research extraction kit from SJ #3 and SJ #6 was used as the donor DNA. Tubes 1-4 were set up as controls to observe for any susceptibility changes to 5E3 with and without antibiotics (tubes 1 and 2), without MRSA genomic DNA (gDNA)(tube 3). The volumes used are listed in Table 4. A 50 µl aliquot from each tube was spread on 2 µg/ml oxacillin Mueller Hinton Agar plates at the

following times: 0 h, 2 h, 4 h, 6 h, 12 h, 24 h, 48 h. Plates were incubated at 37°C for 24 h and observed for growth.

Table 4. Set-up of feasibility study

Tube No.	Mueller Hinton Broth (MHB)	Oxacillin (1 mg/ml)	5E3	MRSA gDNA (24.32 ng/µl)
1	10 ml	--	100 µl	--
2	10 ml	1.25 µl	100 µl	--
3	10 ml	--	100 µl	15 µl
4	10 ml	--	--	--
5	10 ml	1.25 µl	100 µl	15 µl

5E3 inoculum was at a concentration of ~1.5 x 10^5 CFU/ml

3.4.2. Nuclease Test

A nuclease test was employed to detect potential DNA digestion by nucleases produced by MSSA strain 5E3. If the MRSA gDNA added is digested by nuclease produced by the MSSA strain, the uptake of *mecA* may be compromised. Therefore, EDTA was added to prevent DNA digestion. Oxacillin was also added to test any possible effects it may have on nuclease production. The final concentration of oxacillin added to test wells was 0.125 µg/ml ($MIC_{1/2}$ of 5E3). During the initial incubation period, MSSA cells (5E3) were grown in MHB with and without oxacillin for two different time periods. The cells were incubated at 4- and 12 hours to observe any differences in nuclease production. Nucleases produced by the MSSA cells would remain in the supernatant. Therefore, the second step of the nuclease test involved exposure of the supernatant to MRSA gDNA. During the exposure period, EDTA was added to prevent the effects of nuclease digestion.

3.4.2.1. Incubation

An overnight culture of 5E3 was prepared in MHB and diluted to an approximate concentration of 1.5 x 10^5 CFU/ml to inoculate samples. Samples were added to a 96-MicroWell plate. Each well contained 200 µl of MHB and the content of each sample and control wells are shown in Table 5. Two identical sets were prepared for each of the incubation periods, 4- and 12- hours. The cultures were incubated at 37ºC for the indicated period.

Table 5. Nuclease set-up

Well	MHB	5E3	oxacillin (0.1 µg/ml)
1	200 µl	1 µl	--
2	200 µl	--	--
3	200 µl	1 µl	2.50 µl
4	200 µl		2.50 µl

Contents of each well are listed in the table. 1-µl of 5E3 inoculum (~1.5 x 10^5 CFU/ml) was added to the indicated wells. Wells 1 and 3 were to test for the effect of oxacillin on nuclease production. Wells 2 and 4 are controls to test for any contamination in MHB or oxacillin.

3.4.2.2. Exposure to DNA

After incubation, 200 ul of each of the sample was transferred to microcentrifuge tubes and centrifuged for 10 mins at 7,000 x g. The supernatant from each sample was transferred to another set of microcentrifuge tubes. Previously extracted MRSA gDNA (SJ#3 and SJ#6) were added to select tubes. EDTA was also added to observe its effectiveness in preventing DNA digestion. Contents of each tube are shown in Table 6. Tubes were incubated in the shaker for 30 minutes at 37ºC.

Table 6. Sample exposure

Tube	DNA	EDTA (100 mM)	Sampled added
1	10 µl	--	10 µl of Well 1
2	10 µl	--	10 µl of Well 2
3	10 µl	--	10 µl of sterile water
4	10 µl	2 µl	10 µl of Well 1
5	10 µl	--	10 µl of Well 3
6	10 µl	2 µl	10 µl of Well 3
7	--	--	20 µl of Well 1
8	--	--	20 µl of Well3
9	10 µl	--	10 µl of Well 4

Concentration of the stock MRSA gDNA added was 24.3 µg/ml.
Samples were incubated at 37ºC for 30 minutes.

3.4.2.3. Gel electrophoresis

After exposure to DNA, agarose electrophoresis gel (1%) was used to evaluate the samples from Table 6. Each sample (14 µl) was mixed with 6 µl of 1X loading dye (bromophenol blue) and loaded into the comb wells of the gel. A 1kb ladder (5 µl) was used as the standard size ladder. The gel was run for 2 hours at 75 V.

3.4.3. Transformation

3.4.3.1. Test Plates

Mueller Hinton Agar (MHA) (BD, Sparks, MD) plates were prepared containing 2 µg/ml of oxacillin. MHA control plates without oxacillin were also prepared.

3.4.3.2. Transformation Protocols

Three distinct sets of transformation experiments were used in this study. The two variables between the three sets were: 1) the DNA from the same MRSA strains was extracted by two different methods and 2) the timing of oxacillin addition. The first set

(set A) used genomic DNA extracted via the Zymo Research DNA extraction kit with a one-time initial addition of oxacillin to test wells. Comparatively, the second set (set B) used genomic DNA extracted via the phenol/chloroform extraction method also with a one-time initial addition of oxacillin to test wells. The third set (set C) involved the same phenol/chloroform extracted DNA used in set B, however oxacillin was added at each sampling time point to ensure continuous antibiotic pressure. The effects of two different concentrations of EDTA (1 mM and 10 mM) were evaluated in each set. Because the oxacillin level in the culture will decrease due to uptake by the growing bacteria, a concentration of 0.125 μg/ml oxacillin ($MIC_{1/2}$ of oxacillin for 5E3) was maintained by continual addition at each sample time point. An overnight 10 ml broth culture of 5E3 was used to inoculate the test wells. Appropriate positive and negative controls were included. Table 7 represents the general protocol used for each set (A-C). Detailed set-up of the transformation experiment can be found in Appendix B.

Table 7. Set- up for transformation

Well		5E3	MRSA gDNA (~400 ng)	oxa (0.125 µg/ml)	EDTA (1 mM)	EDTA (10 mM)	Treatments
1	Neg ctrl 1	+	-	-	-	-	Control for 5E3 (no additions)
2	Neg ctrl 2A	+	-	+	-	-	Control (antibiotic pressure, no DNA, no EDTA)
3	Neg ctrl 2B	+	-	+	+	-	Control (antibiotic pressure, no DNA, 1mM EDTA)
4	Neg ctrl 2C	+	-	+	-	+	Control (antibiotic pressure, no DNA, 10 mM EDTA)
5	Neg ctrl 3A	+	+	-	-	-	Control (no antibiotic pressure, with DNA, no EDTA)
6	Neg ctrl 3B	+	+	-	+	-	Control (no antibiotic pressure, with DNA, 1 mM EDTA)
7	Neg ctrl 3C	+	+	-	-	+	Control (no antibiotic pressure, with DNA, 10 mM EDTA)
8	Neg ctrl 4	-	-	-	-	-	Control for media (no additions)
9	Test A	+	+	+	-	-	Test (MRSA gDNA and antibiotic pressure)
10	Test B	+	+	+	+	-	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)
11	Test C	+	+	+	-	+	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)

"+" sign denotes addition, "-" sign denotes no addition; 5E3 inoculum was at a stock concentration of 1.5×10^5 CFU/ml. MRSA gDNA was previously extracted from two MRSA strains: SJ #3 and SJ #6.

3.4.3.3. Sampling

A 10-µl aliquot was taken from each test well and dropped on both MHA control plates and MHA containing 2-µg/ml oxacillin plates. Sampling occurred at 7 time intervals: 0, 2, 4, 6, 12, 24, and 48 hours. All inoculated plates were incubated at 37°C for 24 hours and monitored for growth.

3.4.3.4. Colony Forming Units (CFU)

In order to determine the number of cells initially added to each test well, serial dilutions of the overnight inoculum were used to determine the number of colony forming units (CFU). A 5-µl aliquot of the overnight inoculum (representative of the amount added to each test well) used to inoculate test wells was added to 100-µl of MHB and serially diluted (1:100). A 10-µl aliquot of each serial dilution was pipetted onto MHA plates. Plates were incubated at 37°C for 24 hours and colony counts were recorded.

3.4.4. Electroporation

Electroporation is a procedure used to introduce foreign DNA into a cell by increasing the permeability of the cell membrane through the application of an electrical pulse. This procedure was intended to be a positive control for transformation. The same MSSA strain, 5E3, and MRSA genomic DNA (SJ #3 and SJ #6) previously used were applied. Additionally, MSSA genomic DNA (extraction by Zymo kit) from 5E3 was used as a control.

3.4.4.1. Sample preparation

Fifty ml of sterile MHB was inoculated with an overnight broth culture (1:100) of 5E3 and incubated in a shaker at 37°C for 2-4 hours until OD_{550} reached 0.2- 0.9. The cells were harvested by centrifugation at 3000 x g for 5 minutes. The cells were washed with 25 ml of ice-cold 0.5 M sucrose and centrifuged (5 minutes at 3000 x g). Cells were subsequently washed twice with 10 ml of 500-µl of ice-cold 0.5 M sucrose. The final pellet was re-suspended in 200-250-µl of 0.5 M sucrose.

3.4.4.2. Electroporation Procedures

Electroporation provides a method for introducing *mecA* into S.aureus cells. This protocol for electroporation was adapted from Kohler et al., 1997. DNA (200-250ng) was added to 60 µl of washed cells and transferred to 1-mm gap electroporation cuvettes (BTX, Holliston, MA). Cells were electroporated at 1400- 1600 V. One milliliter of sterile MHB was added to each cuvette and was transferred to sterile Eppendorf tubes. The cells were incubated in the shaker at 30°C for 2 hours. Following incubation, 10-100 µl of the culture was spread on a control MHA plate as well as MHA plates containing 2

μg/ml oxacillin. Cultures were further screened on MHA plates containing 6 μg/ml oxacillin + 4% NaCl. The plates were incubated at 37°C for 24 hours and monitored for growth.

3.4.4.3. Colony Polymerase Chain Reaction (PCR)

PCR provides a fast and efficient way to screen for the presence of *mecA* gene complex after electroporation. Isolated colonies were suspended in 20-μl of sterile water and incubated at 95°C for 15 minutes to release DNA content. Following incubation, PCR reactions were set up as previously described in section 3.3.2.2.

Chapter IV. Results

4.1. Bacterial Isolates

In the initial characterization process, mannitol salt fermentation and coagulase production was monitored. A yellow zone surrounding growth on a MSA plate is evidence of mannitol fermentation. Coagulase production is indicated by clotting within the test plasma within 8 hours. Twenty-eight strains of *S. aureus* were tested and the following strains were selected for further characterization: SA 005, SJ #3, SJ #4, SJ #5, SJ #6, 4I2, 5E3, 9D9, 5G8, and BMI-5.Table 8 represents the results of the mannitol and coagulase test for the selected strains. The complete results of these tests can be found in Appendix C.

Table 8 Mannitol fermentation and coagulase test results

	STRAINS	Reference label	Coagulase	Mannitol Fermentation	Classification	Source
1	SA 005	SA 005	+	+	MRSA	Hospital
2	SJ #3	SJ #3	+	+	MRSA	Hospital
3	SJ #4	SJ #4	+	+	MRSA	Hospital
4	SJ #5	SJ #5	+	+	MRSA	Hospital
5	SJ #6	SJ #6	+	+	MRSA	Hospital
6	HMG 218 4I2	4I2	+	+	MSSA	Mouth-guard
7	HMG 207 5E3	5E3	+	+	MSSA	Mouth-guard
8	017 9D9	9D9	+	+	MSSA	N/A
9	HMG 207 5G8	5G8	+	+	MSSA	Mouth-guard
10	BMI-012 D5	BMI-5	+	+	MSSA	Band Musical Instrument

+ sign denotes strain is positive for mannitol fermentation and for coagulase and – sign, negative for mannitol fermentation or coagulase.

4.2. Screening

4.2.1. Susceptibility Test

The MIC levels of oxacillin were determined by the macro-broth dilution method. The results of each trial in addition to the MIC range for each strain are listed in Table 9. Strains that have an MIC level of greater than 4 μg/ml can be classified as MRSA and ones with an MIC level less than 2 μg/ml can qualify as MSSA as per the established interpretive criteria for MIC oxacillin by CLSI (Murray *et al.*, 2009).

Table 9. Minimum inhibitory concentrations of initial strain selection

	MIC level (μg/ml)			
Strain	**Trial 1**	**Trial 2**	**Trial 3**	**MIC range**
SA 005	64	64	32	32-64
SJ #6	>128	128	>128	128+
SJ#5	64	32	32	32-64
SJ #4	64	32	64	32-64
SJ #3	32	64	32	32-64
5E3	0.25	0.25	0.25	0.25
BMI-5	0.5	0.125	0.125	0.125-0.5
9D9	1	0.25	1	0.125-1
5G8	1	0.25	0.25	0.25
4I2	0.5	0.25	0.5	0.25-0.5

*Minimum concentration range (μg/ml) in which there is no visible growth for select MRSA and MSSA strains

4.2.2. Genetic Analysis

DNA concentration of each isolated strain was measured by NanoDrop spectrophotometry. The concentrations measured are indicated in Table 10. The concentrations of DNA were important in the amplification process as well as the

transformation protocol. The additional strains, ATCC 29213 and ATCC 27626 were included as controls in the amplification process.

Table 10. Concentration (ng/ µl) of gDNA extracted from initial selection

	Strains	Conc (ng/ml)
1	SJ #3	42.2
2	SJ #4	44
3	SJ #5	41.5
4	SJ #6	27.3
5	SA005	44.9
6	5E3	43.2
7	9D9	69.4
8	5G8	46.2
9	4I2	27.3
10	BMI-5	43.2
11	ATCC 29213	9.5
12	ATCC 27626	10.3

Combined electropherograms from the genetic analyses revealed the targeted PCR products listed in Table 11. A positive result is registered as a peak in the expected PCR product size range on the electropherogram (see Figure 3 for representative electropherograms). Results of specific note include BMI-5 and 9D9. Referring back to Table 8 with the results of the coagulase and mannitol salt fermentation test, BMI-5 tested positive for coagulase however, the gene was not detected (Table 11). Additionally, MIC tests indicated that 9D9 was sensitive to methicillin and therefore was classified as MSSA, yet *mecA* was detected. Otherwise, the results have been consistent with what was observed.

Table 11. Detection of gene products

	Targeted Gene			
Strain	***BlaZ***	***coA***	***mecA***	***htrA***
SA 005	+	+	+	+
SJ #6	+	+	+	+
SJ #5	+	+	+	+
SJ #4	+	+	+	+
SJ #3	+	+	+	+
5E3	+	+	-	+
BMI-5	+	-	-	+
9D9	+	+	+	+
5G8	+	+	-	+
4I2	+	+	-	+

This table is a compilation of results from electropherograms
"+" denotes the detection of the targeted gene product
"-" denotes the absence of the targeted gene product
Note: BMI-5: positive for phenotypic coagulase test, negative for *coA*
9D9: sensitive to oxacillin but positive for *mecA*

Due to the uncorrelated results, BMI-5 and 9D9 isolates were excluded from further tests. Additional MIC tests were performed on the remaining strains (results not shown). The results of the additional MIC and gene product detection tests were used in selecting candidates for further confirmation and for the transformation experiment. Consequently, MRSA strains SJ #3 and SJ #6 as well as MSSA strains 5E3 and 5G8 were selected.

4.3. Transformation

4.3.1. Confirmation

The MIC levels of the selected strains, MRSA isolates SJ #3 and SJ #6 and MSSA 5E3 and 5G8, were confirmed and are shown in Table 12. MRSA strains SJ #3 yielded a 32 µg/ml average MIC and SJ #6 a MIC range of 32-64 µg/ml. The MIC levels for 5E3 and 5G8 were 0.25 – 0.5 µg/ml and 0.5µg/ml, respectively. Genotypic confirmation of each strain was performed using PCR and subsequent analysis of PCR products on the Genetic Analyzer ABI 310. The results from the electropherograms are summarized in Table 13.

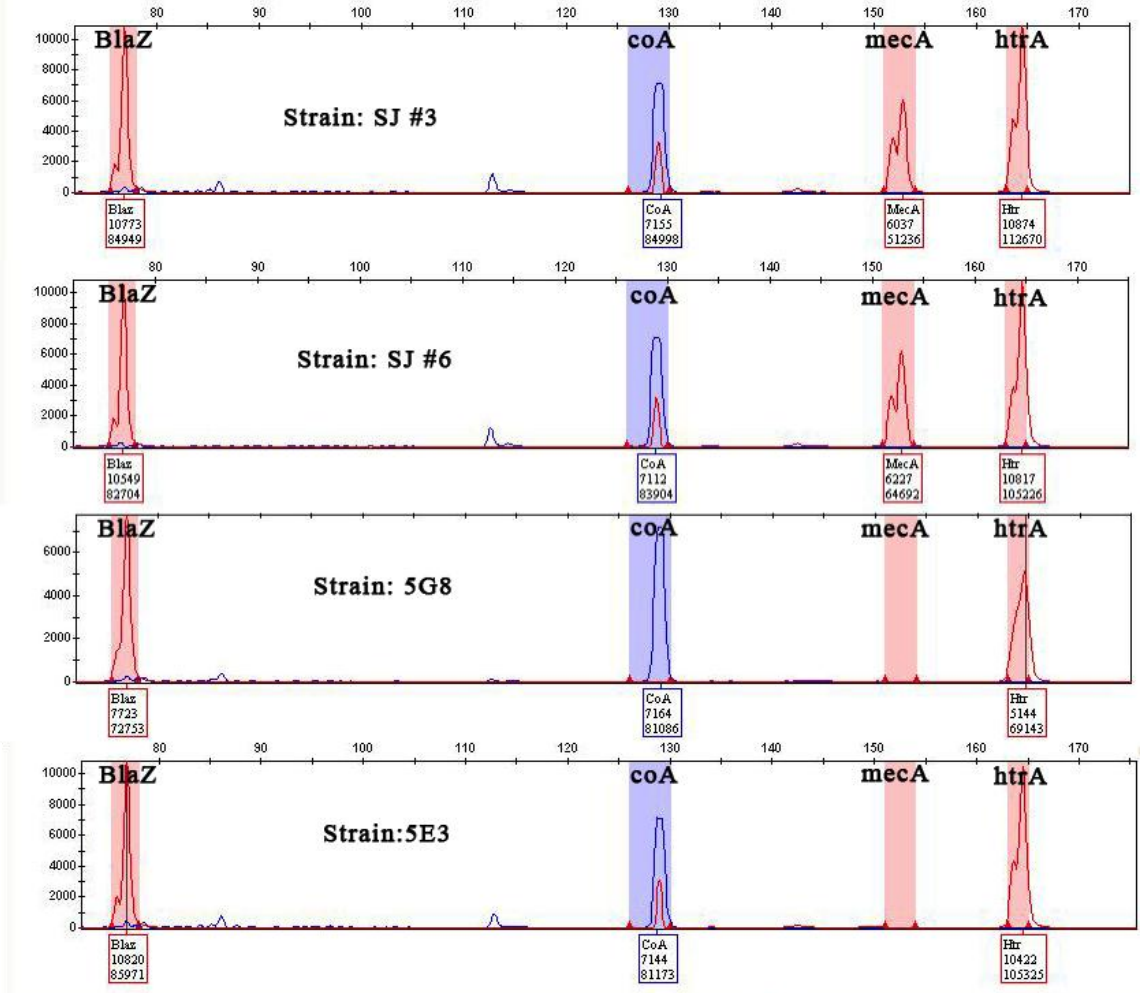
Table 12. Confirmation of MIC values of select MRSA and MSSA strains

	Minimum Inhibitory Concentration (ug/ml)			
Strain	**Trial 1**	**Trial 2**	**Trial 3**	**MIC range**
SJ #6	32	32	64	32-64
SJ #3	32	32	32	32
5E3	0.25	0.5	0.25	0.25-0.5
5G8	0.5	0.5	0.5	0.5

After capillary electrophoresis was completed on the Genetic Analyzer ABI 310, the results were analyzed and visualized using the GeneMapper v3.2 software (Applied Biosystems). Figure 3 shows the representative electropherograms generated for SJ #3, SJ #6, 5G8, and 5E3. Table 13 summarizes the results compiled from the electropherograms for the test strains as well as the quality controls. Amplification of each gene (*BlaZ, coA, mecA,* and *htrA*) was run separately and the PCR products were combined for detection during genetic analysis. In Figure 3, the generated peaks in the electropherograms represent the Relative Fluorescent Units (Y-axis) versus the product

size (X-axis) in basepairs. The fluorescence threshold was set arbitrarily at 400 RFU. Sample products that fluoresced at a value greater than the threshold in addition to falling within the product size range were registered and labeled via GeneMapper v3.2. The quantitative relationship between the magnitudes of fluorescence with the product amount is unknown.

Figure 3. Representative Electropherograms



* Generated peaks in electropherograms represent Relative Fluorescent Units (RFU) (Y-axis) versus the product size. Peaks that were higher than the set threshold (400 RFU) with the correlating product size were registered and labeled via GeneMapper v3.2.

Table 13. Summary of genetic analysis

	Targeted Gene			
Strain	**BlaZ**	**coA**	**mecA**	**htrA**
SJ #3	+	+	+	+
SJ #6	+	+	+	+
5E3	+	+	-	+
5G8	+	+	-	+
ATCC 25293	-	-	-	+
ATCC 27626	+	-	+	-
ATCC 29213	+	+	-	+
Negative Control	-	-	-	-

*ATCC and negative controls included for quality control

Because SJ #3 and SJ #6 had MIC levels greater than 4 µg/ml in addition to being positive for *mecA*, both strains were confirmed to be MRSA and were suitable for subsequent tests. Similarly, 5E3 and 5G8 yielded MIC levels of less than 2 µg/ml and were also negative for *mecA*, therefore were confirmed as MSSA.

4.3.2. Feasibility Test

The feasibility studies confirmed that cells containing the *mecA* gene complex were resistant to 2 µg/ml of oxacillin. Cells that lacked the *mecA* gene complex were susceptible to 2 µg/ml of oxacillin. The main purpose was to test the feasibility of the proposed method for transformation. Revisions to the transformation test were tailored to the results of the feasibility test. MSSA strain 5G8 was used as the recipient strain in this feasibility test and MRSA strains SJ #3 and SJ 6 served as the donor strains. A mixture of SJ #3 and SJ #6 was used. Results of the feasibility test are summarized in Table 14. No growth was observed on screening MHA plates (containing 2 µg/ml oxacillin) at any of the 7 time points (0 – 48 hours) for any of the test samples. The negative results may have been attributed to the high MH broth to DNA volume ratio resulting in a final DNA

concentration of 24.3 ng/ml. Therefore, the volume of MHB was greatly reduced for subsequent transformation experiments.

Table 14. Feasibility test results

Tests	5G8	MRSA gDNA	oxa (0.125 ug/ml)	Purpose	Results on oxacillin (2 ug/ml) plates
Neg ctrl 1	+	-	-	Control for 5G8	No observable growth at any time point (0-48 hours)
Neg ctrl 2	+	-	+	Control for MRSA gDNA	No observable growth at any time point (0-48 hours)
Neg ctrl 3	+	+	-	Control for no antibiotic pressure	No observable growth at any time point (0-48 hours)
Neg ctrl 4	-	-	-	Control for MHB	No observable growth at any time point (0-48 hours)
Test	+	+	+	Observe transfer of MRSA gDNA to MSSA cells	No observable growth at any time point (0-48 hours)

* Sampling at 7 time points (0 – 48 hours) for all test samples yielded no observable growth on test agar plates containing oxacillin.

4.3.3. Nuclease Test

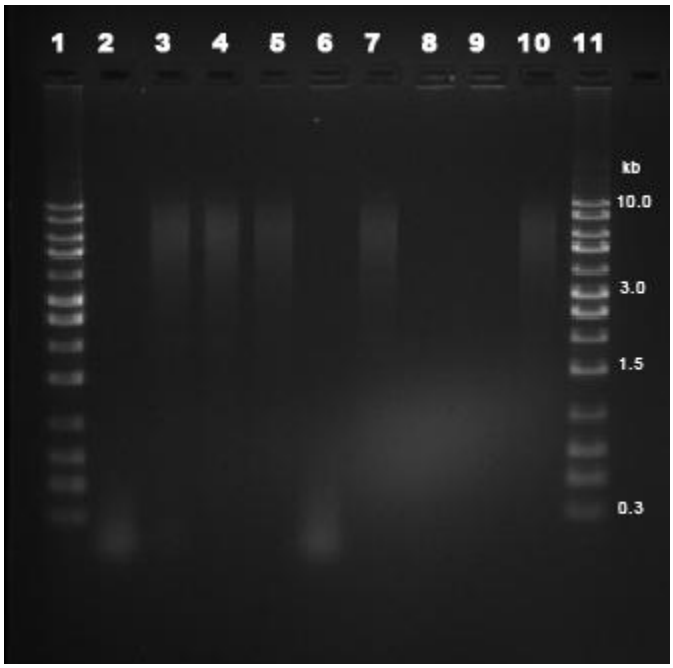
Following incubation at 4- and 12- hours, samples from the nuclease test were loaded on a 1% agarose gel. Table 15 indicates the treatment for the sample placed in each lane of the gels in Figure 4. A 1-kb ladder from Axygen Biosciences (Union City, CA) was used. Figure 4 is the 1% agarose gel of the samples incubated for 4-hours. Only one gel is displayed in Figure 4 because the results generated from 4-hour incubation were also representative of the results from 12-hour incubation. Lanes 2 and 6 contain samples incubated with DNA and with or without oxacillin. Presence of nucleases in the sample was evidenced by

further migration of the digested DNA fragments (<0.3 kb) in lanes 2 and 6. The DNA samples seemed to be unaffected by the addition of oxacillin. Samples containing EDTA (Lanes 5 and 7), however, seemed to prevent the digestion of DNA as migration patterns were similar to control samples containing DNA samples not exposed to nucleases (Lanes 3,4, and 10).

Table 15. Samples loaded into agarose gel

Lane	Sample Treatment
1	1 kb ladder
2	DNA + supernatant (no oxacillin)
3	DNA + MHB (no oxacillin)
4	DNA + sterile water
5	DNA + EDTA + supernatant (no oxacillin)
6	DNA + supernatant (with oxacillin)
7	DNA + EDTA + supernatant (with oxacillin)
8	Supernatant (no oxacillin)
9	Supernatant (with oxacillin)
10	DNA + MHB (with oxacillin)
11	1 kb ladder

Figure 4. Effect of nuclease production on DNA after 4-hour incubation

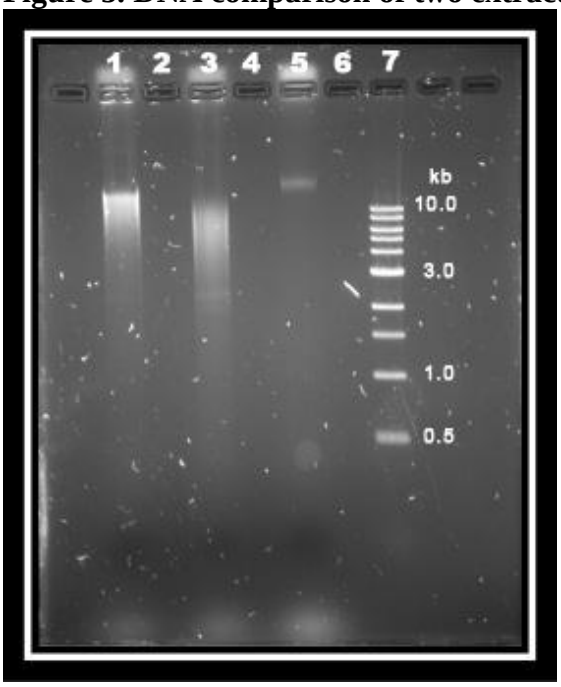


*Refer to Table 15 for samples loaded into each lane of the 1% agarose gels. Digestion of DNA was evident (Lanes 2 and 6). However, oxacillin did not have an observable effect on nuclease production (Lanes 2 and 6). Samples that contained EDTA seemed to prevent digestion of DNA when comparing Lanes 5 and 7 (treated with EDTA) to the control lanes (3, 4, and 10).

4.3.4. DNA integrity

DNA (SJ #3 and SJ#6) extracted using the Zymogen DNA extraction kit was compared to DNA extracted using the phenol: isoamyl: chloroform extraction method. Figure 5 depicts two independently extracted DNA using the Zymo kit in lanes 1 and 3. DNA previously extracted using the phenol:isoamyl:chloroform method was loaded in lane 5 and a 1-kb ladder is depicted in lane 7. The Zymogen-extracted DNA in lanes 1 and 3 showed signs of smearing whereas the phenol-extracted DNA in lane 5 showed a distinct banding pattern.

Figure 5. DNA comparison of two extraction methods



DNA extracted independently using the Zymogen extraction kit (lane 1 and 3) compared to DNA extracted using the phenol extraction method (lane 5) including New England Biolabs 1-kb ladder (Ipswich, MA) (lane 7)

4.3.5. Transformation Experiment

Transformation experiments were performed on three separate occasions to ensure reproducibility and consistency. In the first experiment, no visible growth was noted on the test plates. However, there was growth on the control plates indicating that transformation had not occurred. In the second experiment, no growth was observed on the test plates at 24-hours. The results of the second experiment were excluded due to contamination. The third trial yielded observable results consistent with the first trial. Therefore, no transformation was observed. The complete table of transformation results can be found in Appendix D.

4.4. Electroporation

Electroporation experiments were performed four times with appropriate controls. Brain Heart Infusion (BHI) media was used for the first electroporation experiment. The addition of phenol-extracted MRSA genomic DNA (gDNA) to prepared MSSA cells resulted in colony growth on agar plates containing 2 μg/ml oxacillin. Similar results were observed when Zymo-extracted MRSA gDNA was added to MSSA cells. Negative controls using MSSA gDNA resulted in no growth on oxacillin agar plates. Positive controls using oxacillin-free agar plates were consistently positive. However, the *mecA* gene was not detected by colony PCR in randomly selected colonies from any of the cultures.

In the second electroporation experiment, BHI containing 4% NaCl and 6 μg/ml oxacillin were used as screening plates. When phenol-extracted or Zymo-extracted MRSA gDNA was added to MSSA cells, confluent growth was observed on the screening plates and as well as on BHI plates containing only 2 μg/ml oxacillin. Electroporation of Zymo-extracted MSSA gDNA with MSSA cells yielded no growth on test plates. However, when the MSSA cells were electroporated without any DNA, lawn growth was also observed on test plates. There was growth on control plates.

An additional control was included in the third electroporation experiment. Prepared MSSA cells were electroporated with MRSA gDNA, MSSA gDNA, and with no DNA. The MSSA cells were spread on test plates without electroporation. Lawn growth was observed on all of the plates including the control non-antibiotic agar plates and media plates containing 4% NaCl and 6 μg/ml oxacillin.

The fourth electroporation experiment used different volumes of the plated cells (10, 20, 50, 100 μl). Isolated colonies were observed with each of the electroporation condition. The *mecA* gene was not detected by colony PCR in any of the select colonies

Chapter V. Discussion

The primary objective of this study was to determine the feasibility of transferring *mecA*-driven methicillin resistance to susceptible *S. aureus* under antibiotic pressure. The concept is that susceptible *S. aureus* could gain methicillin resistance by incorporating *mecA*-containing DNA released by MRSA.

5.1. Strain Selection

This study began with an initial pool of 28 clinical strains of *S. aureus*. Each strain was evaluated for the presence of *mecA* gene as well as for resistance to oxacillin by macro-broth dilutions. The selection process was to determine suitable recipients and donors of the *mecA* gene complex.

5.1.1. Minimum Inhibitory Concentrations (MIC)

5.1.1.1. Effect of Salt

MIC was used as a phenotypic indicator and allowed for the quantification of oxacillin resistance levels of *S. aureus*. The effects of salt in the selected growth media were evaluated. The presence of salt resulted in higher MIC levels in MRSA strains, but did not have an effect on the susceptibility of MSSA strains. Thornsberry & McDougal found that the concentration of salt selected for MRSA in heterogenous cultures by an unknown mechanism (Thornsberry & McDougal, 1983).

5.1.2. Genetic Analysis

5.1.2.1. Target Primers

Four sets of primers were used in the evaluation process: *BlaZ, coA, mecA*, and *htrA. BlaZ* codes for resistance to penicillin G. Coagulase production (*coA)* is a major virulence factor specific for *S. aureus* (Johnnson, et al., 1985). The housekeeping gene, *htrA*, is also specific for *S. aureus*.

5.1.2.2. Amplification

Multiplex PCR was initially performed using three genetic markers: *BlaZ, mecA*, and *htrA*. The resultant amplification products were consistent with the individual genotype for each strain and the controls. Attempts to amplify *coA* in multiplex PCR were unsuccessful possible due to competition among the primers of the other three genetic markers. Therefore, singleplex PCR for *coA* had to be performed for each strain and amplicons were then combined for detection.

5.1.2.3. Inconsistent Profiles

During the initial screening process, strains 9D9 and BMI-5 displayed phenotypic profiles inconsistent with their genotypes. MSSA strain 9D9 was sensitive to oxacillin even though *mecA* was detected. This could be due to a mutation in the *mecA* gene that prevents the expression of a functional product. Alternatively, *mecA* may be intact but not expressed in 9D9 for inexplicable reasons. Another strain, BMI-5 tested positive for coagulase; however, *coA* was not detected even after multiple attempts. *coA* was not detected possibly due to a mutation in the target sites preventing primer binding and subsequent amplification.

5.2. Transformation

5.2.1. Feasibility

5.2.1.1. Nuclease Test

The first attempt at transformation was negative, suggesting possible DNA degradation by nucleases. In the nuclease test, ethylenediaminetetraacetic acid (EDTA) was used as a chelating agent to prevent nuclease action. The MSSA culture was incubated for 4- and 12-hours to monitor nuclease production at two different time points with and without the addition of oxacillin. Any nuclease present in the supernatant harvested would degrade the DNA added and be visualized as smearing of DNA staining on an agarose gel. Digestion was evident with gel electrophoresis (Figure 4). The samples that were not treated with EDTA (Lanes 2 and 6) were digested by nucleases as can be visualized on the gel in Figure 4, by the farther migration of the digested DNA fragments. Oxacillin did not seem to have any apparent effects on nuclease production (Lanes 2 and 6). Furthermore, EDTA seemed to prevent the digestion of DNA in the presence of nucleases. Smearing of DNA samples observed in the gel suggested possible shearing of extracted DNA.

5.2.1.2. DNA Extraction

DNA was extracted using the Zymo Research kit and the phenol/chloroform method. The extraction products were loaded on an agarose gel. A slurring pattern was visualized with Zymo-extracted DNA as compared to the distinct bands manifested by the phenol/chloroform-extracted DNA (Figure 5). The Zymo-extraction method involves the rupturing of cells by bead bashing. The DNA degradation

was believed to be due to the mechanical stress placed on the DNA during this extraction process. Therefore, donor MRSA gDNA was extracted by the phenol method.

5.2.2. Transformation

Three transformation experiments were performed with no observed transformation. During the second trial, randomly distributed growth was observed only after 48 h of incubation. Transformation did not occur at 48-hours because the growth was randomly distributed, even on control plates. Also, *mecA* was not detected from the growth. The growth observed was possibly due to the exhaustion of oxacillin in the agar plates by 48 hours therefore permitting growth. The results of the transformation experiment suggest that the conditions or circumstances may not have been favorable for transference to occur. Even under continual antibiotic pressure, transformation was not observed. *In vitro* attempts at interspecies transfer of SCCmec have been unsuccessful in the past (Bloemendaal *et al.*, 2010).

5.2.3. Electroporation

Electroporation is a procedure used to introduce foreign DNA into the cell by increasing the permeability of the cell membrane with the application of an electrical pulse. This procedure was intended to be a positive control to show that 5E3 has the ability to integrate MRSA gDNA. Transformation of MSSA to MRSA using electroporation was not successful after four attempts. Likewise, the *mecA* gene was not detected in any colonies growing on the methicillin test plates.

5.2.4. Interpretation of Results

Although *in vivo* interspecies transfers may have occurred (Bloemendaal *et al.* 2010), transformation may not be associated with *mecA* transfer. Alternatively, conjugation or transduction may be the mechanisms responsible for the observed transfer of methicillin resistance. Ito *et al.* (1999) found that SCC *mecA* cassette lacks the genes for conjugation and transduction. Whether or not these criteria are applicable to all SCC*mec* are unknown. The reason this transformation did not take place may be due to the unique nature of the MRSA gDNA or the MSSA strain, 5E3. With 5 known types of SCC*mec*, ranging in size from 21- to 67-kb, the size of the element may be a factor in transformation with the larger element being more difficult to transfer. Types I, II, and III are most commonly associated with HA-MRSA; whereas Types IV and V are generally found in CA-MRSA. Types IV and V, being significantly smaller in size than the other types (21 kb and 28 kb, respectively), may be more readily incorporated into the bacterial cell genome. (Ito *et al.*, 2004) An indication of the SCC*mec* type in the gDNA extract used in this study could have provided some insight into transference. Alternatively, transformation may have been unsuccessful due to the inability of MSSA 5E3 to incorporate *mecA* into its genome. Integration of SCC*mec* is site-specific on the staphylococcal chromosome. The locus for integration is known as the SCC*mec* attachment site (*attB*). The study of this locus may be important in understanding how receptive a MSSA isolate is to *mecA*. Differences in *attB* sequence of MSSA isolates have suggested the possibility that some MSSA strains may lack the ability to integrate SCC*mec* elements into their chromosomes (Noto *et al.*, 2008). Although EDTA prevented nucleases from degrading donor DNA, it is possible that it may have also

compromised transformation by preventing the uptake or integration of DNA (Noteborn, et al., 1981). The direct effect of EDTA on SCC*mec* and transformation of *S. aureus* may have to be further explored.

5.3. Future Studies

The results of this study indicate that the transference of methicillin resistance between sensitive and resistant strains is a poorly understood process. This study addressed one of the three possible mechanisms by which methicillin-resistance can be transferred, transformation. The inability to accomplish transformation indicates that this process is most likely not the *in vitro* mechanism whereby methicillin resistance is transferred among clinical isolates of *Staphylococcus.* Future studies will address the transference of resistance using either transduction and/or conjugation. Additionally, further analysis of the SCC*mec* and its site specific integration into the genome may be necessary to understand the potential or capacity for transference. Furthermore, using combinations of other *S. aureus* strains or staphylococcal species (e.g. *Staphylococcus epidermidis*) may demonstrate transference of methicillin resistance.

Appendix

Appendix A
ZR Fungal/Bacterial DNA Kit D6005 (modified)

Protocol:

1. Add 50-100 mg fungal or bacterial cells that have been resuspended in up to 200 ul of water or isotonic buffer (e.g. PBS) or up to 200 mg of tissue to a **ZR BashingBead Lysis Tube**. Add 750 ul **Lysis Solution** to the tube.
   ***** Transferred from glass tubes to 15 ml purple top tube and centrifuged for about 3,000 x g , 5 mins. Discard supernatant and resuspend pellet in 200 ul of water, and added to*** *BashingBead Lysis Tube* ********
   *****Transfer (100 ul /sample) DNA elution buffer to microcentrifuge tubes and place in 60°C water bath*****
2. Secure in a Biospec bead beater (Bartlesville, OK), fitted with a 2 ml tube holder assembly and process at maximum speed for 5 minutes
   ***** Balance, secure, set for 3 minutes, turn "on", press "press to start" (yellow button), let rest for 1 minute, press yellow button again*****
3. Centrifuge the **ZR BashingBead Lysis Tube** in a microcentrifuge at ≥10,000 x g for 1 minute.
4. Transfer up to 400 ul supernatant to a **Zymo-Spin IV Spin Filter** (orange top) in a **Collection Tube** and centrifuge at 7,000 rpm (~7,000 x g) for 1 minute.
5. Add 1,200 ul of **Fungal/ Bacterial DNA Binding Buffer** to the filtrate in the **Collection Tube** from Step 4.
6. Transfer 800 ul of the mixture from Step 5 to a **Zymo-Spin IIC Column** in a **Collection Tube** and centrifuge at 10,000 x g for 1 minute.
7. Discard the flow through from the **Collection Tube** and repeat Step 6.
8. Add 200 ul **DNA Pre-Wash Buffer** to the **Zymo-Spin IIC Column** in a new **Collection Tube** and centrifuge at 10,000 x g for 1 minute.
9. Add 500 ul **Fungal/Bacterial DNA Wash Buffer** to the **Zymo-Spin IIC Column** and centrifuge at 10,000 x g for 1 minute.
10. Transfer the **Zymo-Spin IIC Column** to a clean 1.5 ml microcentrifuge tube and add 100 ul **DNA Elution Buffer** directly to the column matrix. *****Let sit for at least 10 minutes***** Centrifuge at 10,000 x g for 30 seconds to elute the DNA.
    **** Set in water bath *60*℃ waterbath****
    Ultra-pure DNA is now ready for use in your experiment.

Appendix B

Transformation trial set-up

Set A: Use *MRSA gDNA from Zymo kit extraction (sheared DNA)

Strain 5E3 (MSSA, MIC 0.25 ug/ml)

Well		MHB	5E3	*MRSA gDNA	oxa (0.125 ug/ml)	EDTA (1 mM)	EDTA (10 mM)	Comments
1	Neg ctrl 1	195.0	5	-	-	-	-	- ctrl for transference, + ctrl for growth
2	Neg ctrl 2A	192.5	5	-	2.5	-	-	- ctrl (antibiotic pressure, no DNA)
3	Neg ctrl 2B	190.5	5	-	2.5	2	-	- ctrl (antibiotic pressure, no DNA, 1mM EDTA)
4	Neg ctrl 2C	191.3	5	-	2.5	-	1.2	- ctrl (antibiotic pressure, no DNA, 10 mM EDTA)
5	Neg ctrl 3A	179.0	5	16	-	-	-	- ctrl (no antibiotic pressure, with DNA)
6	Neg ctrl 3B	177.0	5	16	-	2	-	- ctrl (no antibiotic pressure, with DNA, 1 mM EDTA)
7	Neg ctrl 3C	177.8	5	16	-	-	1.2	- ctrl (no antibiotic pressure, with DNA, 10 mM EDTA
8	Neg ctrl 4	200.0	-	-	-	-	-	- ctrl for growth in media
9	Test A	176.5	5	16	2.5	-	-	Test with MRSA gDNA and antibiotic pressure
10	Test B	174.5	5	16	2.5	2	-	Test with MRSA gDNA, antibiotic pressure, 1 mM EDTA
11	Test C	175.3	5	16	2.5	-	1.2	Test with MRSA gDNA, antibiotic pressure, 10 mM EDTA

Stock solutions

MRSA gDNA = 24.3 ng/ul

oxacillin = 0.01 mg/ml

EDTA (1 mM)= 100 mM

EDTA(10 mM) = 250 mM

All volumes added were in microliters (ul). An overnight culture of MSSA strain 5E3 was used to inoculate.

Appendix B

Transformation trial set-up (continued)

Set B: Use *MRSA gDNA (SJ #3 and SJ #6) from phenol extraction

Strain 5E3 (MSSA, MIC 0.25 ug/ml)

Well		MHB	5E3	*MRSA gDNA	oxa (0.125 ug/ml)	EDTA (1 mM)	EDTA (10 mM)	Comments
1	Neg ctrl 1	195.0	5	-	-	-	-	- ctrl for transference, + ctrl for growth
2	Neg ctrl 2A	192.5	5	-	2.5	-	-	- ctrl (antibiotic pressure, no DNA)
3	Neg ctrl 2B	190.5	5	-	2.5	2	-	- ctrl (antibiotic pressure, no DNA, 1mM EDTA)
4	Neg ctrl 2C	191.3	5	-	2.5	-	1.2	- ctrl (antibiotic pressure, no DNA, 10 mM EDTA)
5	Neg ctrl 3A	189.0	5	6	-	-	-	- ctrl (no antibiotic pressure, with DNA)
6	Neg ctrl 3B	187.0	5	6	-	2	-	- ctrl (no antibiotic pressure, with DNA, 1 mM EDTA)
7	Neg ctrl 3C	187.8	5	6	-	-	1.2	- ctrl (no antibiotic pressure, with DNA, 10 mM EDTA
8	Neg ctrl 4	200.0	-	-	-	-	-	- ctrl for growth in media
9	Test A	186.5	5	6	2.5	-	-	Test with MRSA gDNA and antibiotic pressure
10	Test B	184.5	5	6	2.5	2	-	Test with MRSA gDNA, antibiotic pressure, 1 mM EDTA
11	Test C	185.3	5	6	2.5	-	1.2	Test with MRSA gDNA, antibiotic pressure, 10 mM EDTA

Stock solutions

MRSA gDNA = 70 ng/ul

oxacillin = 0.01 mg/ml

EDTA (1 mM)= 100 mM

EDTA (10 mM) = 250 mM

All volumes added were in microliters (ul). An overnight culture of MSSA strain 5E3 was used to inoculate.

Appendix B

Transformation set-up (continued)

Set C: Used *MRSA gDNA (SJ #3 and SJ #6) from phenol extraction - Continuous antibiotic pressure

Strain: 5E3 (MSSA, MIC 0.25 ug/ml)

Well		MHB	5E3	*MRSA gDNA	oxa (0.125 ug/ml)	EDTA (1 mM)	EDTA (10 mM)	Comments
1	Neg ctrl 1	195.0	5	-	-	-	-	- ctrl for transference, + ctrl for growth
2	Neg ctrl 2A	192.5	5	-	2.5	-	-	- ctrl (antibiotic pressure, no DNA)
3	Neg ctrl 2B	190.5	5	-	2.5	2	-	- ctrl (antibiotic pressure, no DNA, 1mM EDTA)
4	Neg ctrl 2C	191.3	5	-	2.5	-	1.2	- ctrl (antibiotic pressure, no DNA, 10 mM EDTA)
5	Neg ctrl 3A	189.0	5	6	-	-	-	- ctrl (no antibiotic pressure, with DNA)
6	Neg ctrl 3B	187.0	5	6	-	2	-	- ctrl (no antibiotic pressure, with DNA, 1 mM EDTA)
7	Neg ctrl 3C	187.8	5	6	-	-	1.2	- ctrl (no antibiotic pressure, with DNA, 10 mM EDTA
8	Neg ctrl 4	200.0	-	-	-	-	-	- ctrl for growth in media
9	Test A	186.5	5	6	2.5	-	-	Test with MRSA gDNA and antibiotic pressure
10	Test B	184.5	5	6	2.5	2	-	Test with MRSA gDNA, antibiotic pressure, 1 mM EDTA
11	Test C	185.3	5	6	2.5	-	1.2	Test with MRSA gDNA, antibiotic pressure, 10 mM EDTA

Stock solutions

MRSA gDNA = 70 ng/ul

oxacillin = 0.01 mg/ml

EDTA (1 mM)= 100 mM

EDTA (10 mM) = 250 mM

All volumes added were in microliters (ul). An overnight culture of MSSA strain 5E3 was used to inoculate.

Appendix C
Initial Characterization of *S. aureus* strains

	STRAINS	Reference label	Coagulase	Mannitol Fermentation	Classification	Source
1	SA 001	SA 001	+	+	MRSA	Hospital
2	SA 002	SA 002	+	+	MRSA	Hospital
3	SA 003	SA 003	+	+	MRSA	Hospital
4	SA 004	SA 004	+	+	MRSA	Hospital
5	SA 005*	SA 005	+	+	MRSA	Hospital
6	SA 006	SA 006	-	-	MRSA	Hospital
7	SA 007	SA 007	+	+	MRSA	Hospital
8	SA 008	SA 008	+	+	MRSA	Hospital
9	SA 009	SA 009	+	+	MRSA	Hospital
10	SA 010	SA 010	+	+	MRSA	Hospital
11	SA 011	SA 011	+	+	MRSA	Hospital
12	SJ #1	SJ #1	+	-	MRSA	Hospital
13	SJ #2	SJ #2	+	+	MRSA	Hospital
14	SJ #3*	SJ #3	+	+	MRSA	Hospital
15	SJ #4*	SJ #4	+	+	MRSA	Hospital
16	SJ #5*	SJ #5	+	+	MRSA	Hospital
17	SJ #6*	SJ #6	+	+	MRSA	Hospital
18	SJ #7	SJ #7	+	+	MRSA	Hospital
19	HMG 218 4I2	4I2	+	+	MSSA	Mouthguard
20	HMG 207 5E3	5E3	+	+	MSSA	Mouthguard
21	HMG 218 4I3	4I3	-	-	MSSA	Mouthguard
22	FMP 143-46 2A9	2A9	-	-	MSSA	Mouthguard
23	HMG 213 4I1	4I1	-	+	MSSA	Mouthguard
24	014 10H1	10H1	-	-	MSSA	N/A
25	017 9D9	9D9	+	+	MSSA	N/A
26	HMG 207 5G8	5G8	+	+	MSSA	Mouthguard
27	BMI-012 D5	BMI-5	+	+	MSSA	Band Musical Instrument
28	HMG 206 6A7	6A7	-	+	MSSA	Mouthguard

"+"- sign denotes a positive result for mannitol fermentation or coagulase
"-"-sign denotes a negative result for mannitol fermentation or coagulase

Appendix D

Complete Transformation (Trials 1-3) Results

Transformation results: Trial 1

Set A: Used *MRSA gDNA from Zymo kit extraction

Well	Treatment	0 h		2 h		4 h		6 h		12 h		24 h		48 h	
		Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	MHA	Ctrl	Oxa MHA	Ctrl	MHA	Ctrl	MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin

Set B: Used *MRSA gDNA from phenol extraction

Well	Treatment	0 h		2 h		4 h		6 h		12 h		24 h		48 h	
		Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MH	Ctrl	Oxa MHA	Ctrl	Oxa MH	Ctrl	Oxa MH	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin

Set C: Used MRSA gDNA from phenol extraction- Continual antibiotic pressure

Well	Treatment	0 h		2 h		4 h		6 h		12 h		24 h		48 h	
		Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin

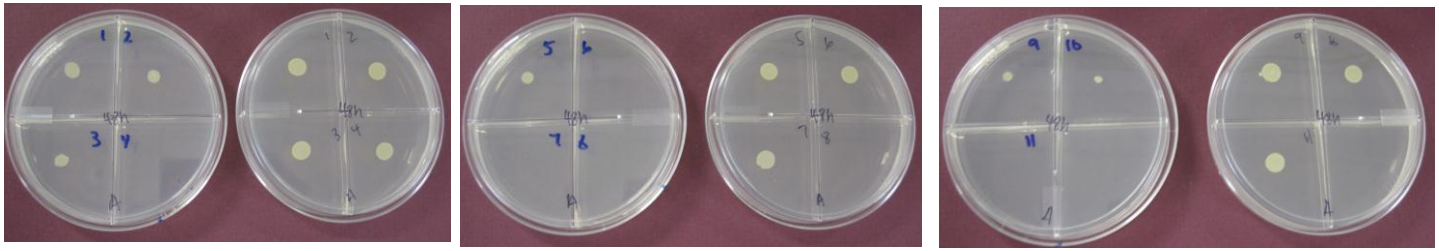
No representative photos available for trial 1

Appendix D continued

Transformation results: Trial 2

Set A: Used *MRSA gDNA from Zymo kit extraction		0 h		2 h		4 h		6 h		12 h		24 h		48 h	
Well	Treatment	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin



48 hour plates- Observed growth on 1, 2, 3,5, 9, 10 (MHA + 2 ug/ml plates)

Representative plates on the right of each photo is an MHA plate without oxacillin, plate on the left of each photo is an MHA plate containing 2 µg/ml of oxacillin.

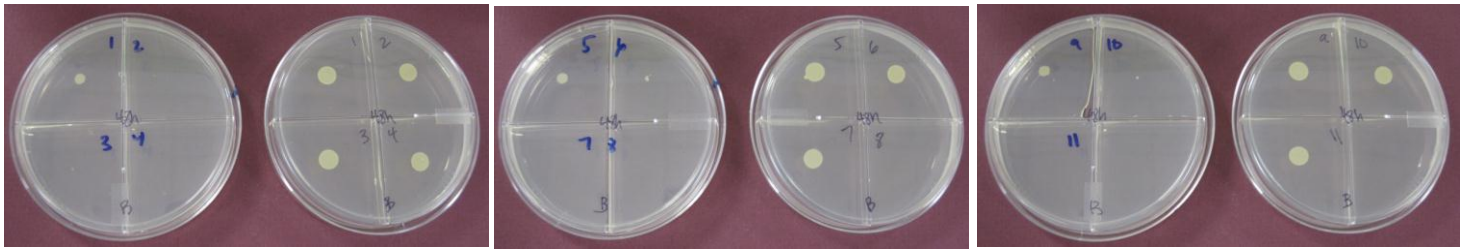
Appendix D continued

Transformation results: Trial 2

Set B: Used *MRSA gDNA from phenol extraction

		0 h		2 h		4 h		6 h		12 h		24 h		48 h	
Well	Treatment	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin



48 hour plates: Observable growth on 1, 5, 6, 9, 10

Representative plates on the right of each photo is an MHA plate without oxacillin, plate on the left of each photo is an MHA plate containing 2 µg/ml of oxacillin.

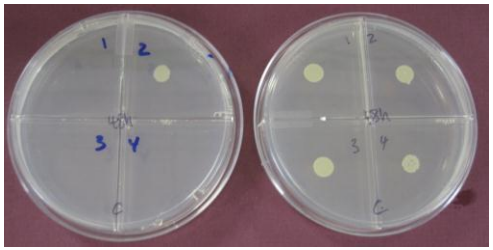
Appendix D continued

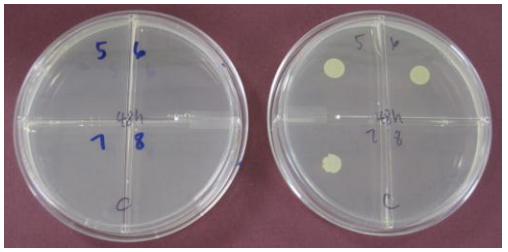
Transformation results: Trial 2 (continued)

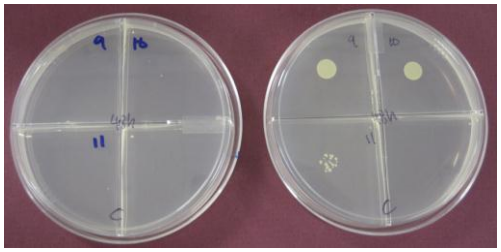
Set C: Used MRSA gDNA from phenol extraction- Continual antibiotic pressure

		0 h		2 h		4 h		6 h		12 h		24 h		48 h	
Well	Treatment	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	+	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin







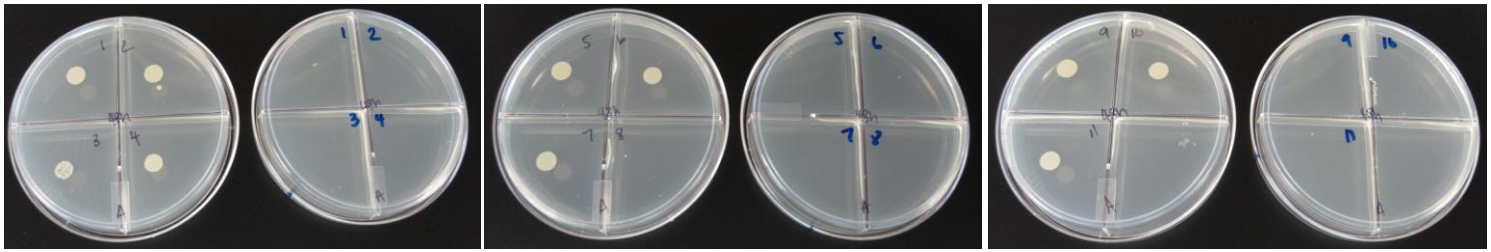
Representative plates on the right of each photo is an MHA plate without oxacillin, plate on the left of each photo is an MHA plate containing 2 µg/ml of oxacillin.

Appendix D continued

Transformation results: Trial 3

Set A: Used *MRSA gDNA from Zymo kit extraction		0 h		2 h		4 h		6 h		12 h		24 h		48 h	
Well	Treatment	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin



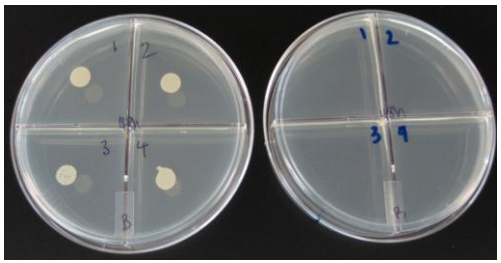
Representative plates on the right of each photo is an MHA plate without oxacillin, plate on the left of each photo is an MHA plate containing 2 µg/ml of oxacillin.

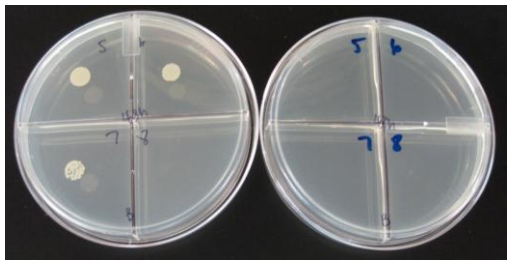
Appendix D Continued

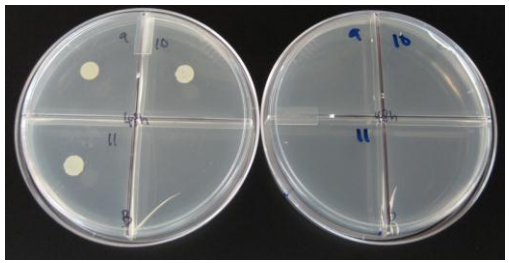
Transformation results: Trial 3 (continued)

Set B: Used *MRSA gDNA from phenol extraction		0 h		2 h		4 h		6 h		12 h		24 h		48 h	
Well	Treatment	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin







Representative plates on the right of each photo is an MHA plate without oxacillin, plate on the left of each photo is an MHA plate containing 2 µg/ml of oxacillin.

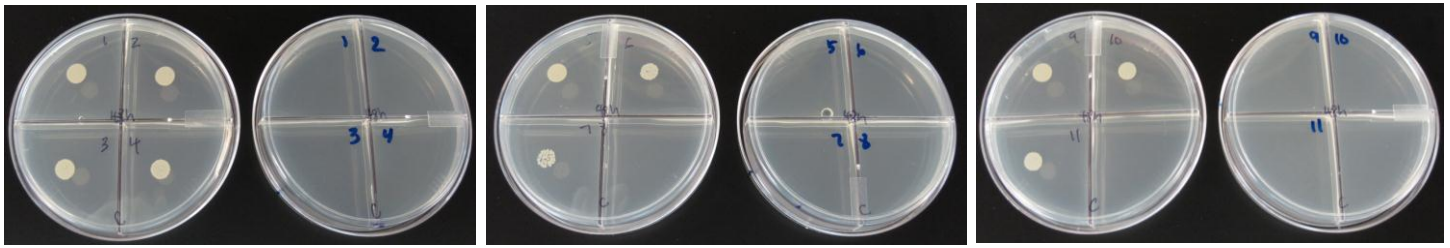
Appendix D Continued

Transformation results: Trial 3 (continued)

Set C: Used MRSA gDNA from phenol extraction- Continual antibiotic pressure

		0 h		2 h		4 h		6 h		12 h		24 h		48 h	
Well	Treatment	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl	Oxa MHA	Ctrl
1	Control for 5E3 (no additions)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
2	Control (antibiotic pressure, no DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
3	Control (antibiotic pressure, no DNA, 1mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
4	Control (antibiotic pressure, no DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
5	Control (no antibiotic pressure, with DNA, no EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
6	Control (no antibiotic pressure, with DNA, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
7	Control (no antibiotic pressure, with DNA, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
8	Control for media (no additions)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Test (MRSA gDNA and antibiotic pressure)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
10	Test (MRSA gDNA, antibiotic pressure, 1 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+
11	Test (MRSA gDNA, antibiotic pressure, 10 mM EDTA)	-	+	-	+	-	+	-	+	-	+	-	+	-	+

(+) indicates growth, (-) indicates no growth Oxa MHA- Mueller Hinton Agar plates contained 2 ug/ml oxacillin



Representative plates on the right of each photo is an MHA plate without oxacillin, plate on the left of each photo is an MHA plate containing 2 µg/ml of oxacillin

Chapter VI. References

Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy,* 48, 5-16.

Archer, G. L., Niemeyer, D.M. (1994). Origin and evolution of DNA associated with resistance to methicillin in *Staphylococci*. *Trends Microbiol, 2*, 343-347.

Arias, C. A., Murray, B.E. (2009). Antibiotic-Resistant Bugs in the 21st Century--A Clinical Super-Challenge. *N Engl J Med, 360*, 439-443.

Boyle-Vavra, S., Daum, R.S. (2007). Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leukocidin. *Laboratory Investigation, 87*, 3-9.

Bloemendaal, A.L.S., Brouwer, E.C., Fluit,A.C. (2010). Methicillin resistance transfer from *Staphylococcus epidermidis* to methicillin-susceptive *Staphylococcus aureus* in a patient during antibiotic therapy. *PLoS ONE*, 5(7).

Center for Disease Control and Prevention (2011). Methicillin-resistant *Staphylococcus aureus* (MRSA). Retrieved May 2, 2011, 2011, from http://www.cdc.gov/mrsa/index.html

Chambers, H. (2001). The changing epidemiology of *Staphylococcus aureus*. *Emerging Infectious Diseases, 7*(2), 178-182.

Chen, I., Dubnau, D. (2004). DNA uptake during bacterial transformation. *Nature Reviews, 2*, 241-249.

Demling, R. H., Waterhouse, B. (2007). The increasing problem of wound bacterial

burdern and infection in acute and chronic soft-tissue wounds caused by methicillin-resistant *Staphylococcus aureus*. *Journal of Burns and Wounds, 7,* 86-98.

Dugdale, D. V., Vyas, J.M. (2009). MRSA. Retrieved July 14, 2011, from http://www.nlm.nih.gov/medlineplus/ency/article/007261.htm

Ender, M., McCallum, N., Adhikari, R., Berger-Bachi, B. (2004). Fitness Cost of SCC*mec* and methicillin resistance elvels in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 48*(6), 2295-2297.

Enright, M. C., Robinson, A.D., Gaynor, R., Feil, E.J., Grundmann, H., Spratt, B.G. (2002). The evolutionary history of methicilin-resistant *Staphyococcus aureus* (MRSA). *PNAS, 99*(11), 7687-7692.

Glass, R. T., Conrad, R.S., Kohler, G.A., Bullard, J.W. (2011). Evaluation of the microbial flora found in woodwind and brass instruments and their potential to transmit diseases. *General Dentistry*, 59(2), 100-107

Glass, R. T., Conrad, R.S., Wood, C.R., Warren, A.J., Kohler, G.A., Bullard, J.W., Benson, G., Gulden, J.M. (2009). Protective Athletic Mouthguards: Do they cause harm? *Sports Health, 1*, 411-415.

Goetghebeur, M., Landry, P.A., Han, D., Vicent, C. (2008). Methicillin-resistant *Staphylococcus aureus*: A public health issue with economic consequences. *Can J Infect Dis Med Microbiol*. *18*(1), 27-34.

Griethuysen, A. V., Loo, I.V., Belkum, A.V., Vandenbroucke-Grauls, C., Wannet, W., Keulen, P.V., Kluytmans, J. (2005). Loss of the *mecA* gene during storage of methicillin-resistant *Staphylococcus aureus* strains. *Journal of Clinical Microbiology, 43*(3), 1361-1365.

Hanssen, A. M., Sollid, J.U.E. (2006). SCC*mec* in Staphylococci: Genes on the move. *FEMS Immunol Med Microbiol, 46*, 8-20.Katayama, Y., Ito,T., Hiramatsu, K. (2000). A New Class of Genetic Element, Staphylococcus Cassette Chromosome *mec*, Encode Methicillin Resistance in *Staphylococcus*. *Antimicrobial Agents and Chemotherapy, 44*(6), 1549-1555.

Ito, T., Katayama Y., Hiramatsu, K. (1999). Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrobial Agents and Chemotherapy, 43*, 1449-1458.

Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H., Hiramatsu, K. . (2004). Novel Type V Staphylococcal Cassette Chromosome *mec* Driven by a Novel Cassette Chromosome Recombinase *ccrC*. *Antimicrobial Agents and Chemotherapy, 48*(7), 2637-2651.

Johnsson, P., Lindberg, M., Haraldsson, I., Wadstrom, T. (1985). Virulence of *Staphylococcus aureus* in Mouse Mastitis Model Studies of alpha hemolysin, coagulase, and protein A as possible virulence determinants with protoplast fusion and gene cloning. *Infection and Immunity, 49*(3), 765-769.

Jones, M. E., Koehrer, K., Schmitz, F.J. (1996). Multiplex PCR for the Rapid Simultaneous Speciation and Detection of Methicillin-Resistance and Genes Encoding Toxin Production in *Staphylococcus aureus*. In S.H. Gillepsie (Ed.), Antibiotic Resistance Methods and Protocols (pp. 103-112). New Jersey: Humana Press.

Köhler GA, White TC, Agabian N. Overexpression of a cloned IMP dehydrogenase gene of *Candida albicans* confers resistance to the specific inhibitor mycophenolic acid. *J Bacteriol*, 179(7), 2331-2338 (1997).

Lu, P. L., Chin, L.C., Peng, C.F., Chiang, Y.H., Chen,T.P., Ma, L., Siu, L.K. (2005). Risk factors and molecular analysis of community methicillin-resistant *Staphylococcus aureus* carriage. *Journal of Clinical Microbiology, 43*(1), 132-139.

McClane, B. A., Mietzner, T.A., Dowling, J.N., Phillips, B.A. (1999). *Microbial Pathogenesis* (1 ed.). Madison, CN: Fence Creek Publishing.

McDougal, L. K., Thornsberry, C.K. (1986). The role of beta-lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *Journal of Clinical Microbiology, 23*(5), 832-839.

Murray, P. R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R.H. (2008). *Manual of Clinical Microbiology* (9 ed.): ASM Press

Noteborn M., V., G., Kooistra, J. (1981). Effect of ethylendiaminetetraacetic acid on deoxyribonucleic acid entry and recombination in the transformation of a wild-type strain and a *rec-1* mutant of *Haemophilus influenzae*. *Journal of Bacteriology, 145*(3), 1180-1195.

Noto, M. J., Kreiswirth, B.N., Monk, A.B., Archer, G.L. (2008). Gene Acquisition at the Insertion Site for SCC*mec*, the Genomic Island Conferring Methicillin Resistance in *Staphylococcus aureus*. *Journal of Bacteriology*, *190*(4), 1276-1283.

Parker, M. T., Hewitt, J.H. (1970). Methicillin resistance in *Staphylococcus aureus*. *The Lancet, 295* (7651), 800-804.

Petersson, A. C., Kamme, C., Miorner, H. (1999). Disk with high oxacillin content discriminates between methicillin-resistant and borderline methicillin-susceptible *Staphylooccus aureus* strains in disk diffusion assays using a low salt concentration. *J Clin Microbiol, 37*(6), 2047-2050.

Thornsberry, C., McDougal, L.K. (1983). Successful use of broth microdiluation in susceptibility tests for methicillin-resistant (heteroresistant) Staphylococci. *Journal of Clinical Microbiology, 18*(5), 1084-1091.

Tomasz, A., Drugeon, H.B., Lencastre, H.M.D., Jabes, D., McDougal, L., Bille, J. (1989). New mechanism for the methicillin resistance in *Staphylococcus aureus*: Clinical Isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin binding capacity. *Antimicrobial Agents and Chemotherapy*, *33*(11), 1869-1874.

Walsh, Christopher. (2003) Antibiotics: Actions, origins, resistance. Washington, DC: American Society of Microbiology Press.

Wielders, C. L. C., Fluit, A.C., Brisse, S., Verhoef, J., Schmitz, F.J. (2002). *mecA* Gene is widely disseminated in *Staphylococcus aureus* population. *Journal of Clinical Microbiology*, *40*(11), 3970-3975.

Wielders, C. L. C., Vriens, M.R., Brisse, S., de Graaf-Miltenburg, L.A.M., Troelstra, A., Fleer, A., Schmitz, F.J., Verhoef, J., Fluit, A.C. (2001). Evidence for *in-vivo* transfer of mecA DNA between *staphylococci*. *The Lancet, 357,* 1674- 1675.

Wing, R. W., Kulkarni, R. (2010). Staphylococcal Scalded Skin Syndrome in Emergency Medicine. Retrieved July 14 2011, from http://emedicine.medscape.com/article/788199-overview

VITA

Trang Xuan Pham

Candidate for the Degree of

Master of Science

Thesis: TRANSFERENCE OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* USING ANTIBIOTIC CHALLENGE

Major Field: Forensic Science

Education:

Completed the requirements for the Master of Science in Forensic Sciences at Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma, in December, 2011

Completed the requirements for the Bachelor of Science in Biochemistry at Wichita State University, Wichita, Kansas, in 2008.

Name: Trang Pham Date of Degree: December, 2011

Institution: Oklahoma State University Center for Health Sciences Location: Tulsa, Oklahoma

Title of Study: TRANSFERENCE OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* USING ANTIBIOTIC CHALLENGE

Pages in Study: 66 Candidate for the Degree of Master of Science

Major Field: Forensic Sciences

Scope and Method of Study: The specific aim of this study was to document the transfer of a functional methicillin resistance gene, *mecA*, from methicillin resistant *Staphylococcus aureus* (MRSA) genomic DNA to methicillin sensitive *Staphylococcal aureus* (MSSA) thereby converting MSSA to MRSA. The experimental approach included the characterization of *Staphylococcus aureus* strains using Gram stain, coagulase and mannitol salt agar tests. Included among the *Staphylococcus* strains analyzed were 18 de-identified MRSA hospital isolates, nine MSSA strains isolated from band musical instruments and mouth-guards studies, and one MSSA strain of unknown origin. Additionally, sensitivity profiles of each strain were determined by minimum inhibitory concentration (MIC). Detection of *mecA* gene involved amplification by polymerase chain reaction (PCR) using fluorescently tagged primers and capillary electrophoresis with an ABI Prism Genetic Analyzer 310. Transformation studies involved the suspension of DNA extracted from two MRSA strains into a MSSA culture containing oxacillin complete with necessary controls. Electroporation was used in an attempt to force transformation, but it too was unsuccessful.

Findings and Conclusions: Transformation was not observed under the specific test conditions. As a result of the electroporation experiment, growth was observed but no *mecA* gene was detected. The difficulty in transforming MSSA to MRSA suggests that transformation of SCC*mec* containing a *mecA* gene may not readily occur in nature or if so, it may proceed under conditions that were not replicated *in vitro*. Furthermore, additional analysis of *SCC*mec containing a *mecA* gene of the *Staphylococcal* strains may be necessary to further assess the integration of *mecA* into a recipient's genome. Lastly, transference of *mecA* to MSSA may be a result of conjugation or transduction, not transformation.

ADVISER'S APPROVAL: Dr. Tom Glass