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Characterization and Antibiotic Resistance Profile of Staphylococci in The Lower Rio Grande Valley

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CHARACTERIZATION AND ANTIBIOTIC RESISTANCE

PROFILE OF STAPHYLOCOCCI IN THE

LOWER RIO GRANDE VALLEY

A Thesis

by

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ABSTRACT

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Staphylococci from part of the natural flora of humans and some, like *Staphylococcus aureus,* appear to be evolving antibiotic resistance. The Lower Rio Grande Valley (LRGV) is an area of interest due to its proximity to the US/Mexico border where antibiotics could recently be purchased without prescription. Two libraries from community association (CA-S) and environmental association (E-S) have been collected and the staphylococci isolated. We hypothesize that their SCC*mec* type, which aids the staphylococci in antibiotic resistance, will not display classically defined association schemes and will surface in many species of staphylococci. This study generates antibiotic resistance profiles via antibiotic susceptibility testing, identifies the presence of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, the presence of the *mecA* gene, and SCC*mec* types I-V among both libraries through multiplex and standard polymerase chain reaction (PCR). The data produced is an insight into the composition of the staphylococci in the LRGV.

DEDICATION

Before anything else, I give thanks to my Lord and Savior for always listening to and answering my prayers. This thesis is dedicated to my family. Without your unwavering support and love, the completion of my master's studies would certainly not have been realized.

Para mis padres, Argelio Treviño y Diana Betancourt Treviño, gracias por los valores que han inculcado en mí y por ser el ejemplo de que hay que trabajar duro y seguir trabajando para realizar nuestros sueños. Siempre han dicho que están muy orgullosos de mí, pero por sus sacrificios y el cariño que han tenido con nosotros, soy yo quien está orgullosa y honrada de poder llamarles mis padres.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

Staphylococcus

Staphylococcus is a genus of gram-positive bacteria that are characteristically cocci in morphology (33). There are currently 41 species identified in the genus (13). They grow in grape-like cellular clusters on the skin and mucosal membranes of mammals and few thermo regulating species (33). In addition, staphylococci have also been known to grow on improperly refrigerated food which, if ingested, can cause food poisoning (33).

Two particular types of Staphylococci, *Staphylococcus aureus* and *Staphylococcus epidermidis* are closely associated with humans. Humans are often carriers of these two organisms. *S. aureus* thrives and breeds rapidly in the presence of CO_2, therefore, it is easily understandable that it resides in the nares and throats of individuals (13). At birth, all humans are exposed to *S. aureus* (13). Into adulthood though, 20-50 % of individuals will have persistent nosocomial colonization of the organism (13). *S. epidermidis* lives more readily on skin due to its ability to tolerate saline and acidic environments, as well as survive in the presence of lipids (13).

These species are also largely the origin of infection in humans which highlight the significance of the two. *S. aureus* and *S. epidermidis* have historically been organisms pathogenic to humans causing a variety of complications. *S. auerus*, for example, can cause diseases as severe as septic arthritis, necrotizing pneumonia, and septic shock (13). These conditions can worsen in immunocompromised individuals which can lead to mortality (13). A threat to even non-immunocompromised individuals is that of methicillin-resistant *Staphylococcus aureus* (MRSA) which harbors resistance to β-lactam antibiotics as well as multiple classes of antibiotics and thus, is difficult to treat should an infection from such an organism become established (33).

S. epidermidis is the most predominant species found on the human epidermis (13). The species can commonly cause bacteremia and have been known to cause complications due to infections of surgical sites or medical implant sites (13). The pathogenicity that the species harbors is due to its ability to produce and establish a biofilm, and similarly to *S. aureus*, its ability to acquire antibiotic resistance (13).

Staphylococcus haemolyticus is found readily on humans and animals such as primates and some domestic animals (13). In humans, it grows on axillae, inguinal, and perineum areas (13). *S. haemolyticus* has been known to cause several infections in humans including urinary tract infections and orthopedic infections (13). This is an organism of interest because it has incorporated in its genome several virulence genes along with resistance to multiple antibiotics (13).

The study presented herein focused on the three previously mentioned species as *S.auerus* and *S.epidermidis* are commonly occurring in humans at a high frequency and *S. haemolitycus* has a prominent history for harboring multiple antibiotic resistance.

Antibiotics

Antibiotics, also known as antimicrobials, are chemical compounds that limit or eradicate microbial organisms such as bacteria and fungi (44). Antibiotics can display two modes of controlling microbial growth. One of these is by bactericidal mechanisms in which bacterial cells die (44). The other is bacteriostatic, in which bacterial growth is arrested (44). This eventually leads to a bactericidal outcome.

Antibiotics can come from two sources, one of which is naturally occurring while the other is synthetically made (9). Naturally made antibiotics are a product of several types of bacteria and fungi (9). *Actinomycetes* are the genus of bacteria that produce a majority of antibiotics in natural settings (44). These antibiotic compounds are produced by the bacteria in response to competition in the environment (44). They are created to help the bacteria completely eliminate or slow the growth of nearby microorganisms (44).

Synthetically produced antibiotics involve chemical derivation (44). Some manufactured antibiotics are also semi-synthetic derivatives in general. They have a bacterial origin similar to naturally occurring antibiotics, yet they are optimized for efficacy with chemical or genetic manipulation (44). Three classes of synthetically manufactured antibiotics are commonly used in the clinical setting. These are sulfa drugs, quinolones, and oxazolidinones (44).

Currently, there are four major classes of antibiotics; however, there are over one hundred different types of antibiotics (20, 44). The classes of antibiotics are determined based on

3

their bacterial target site whether it is on the cell surface or through an inner cell mechanism (20). The four modes of action of antibiotics are through inhibition or disruption of the following: cell wall biosynthesis, protein biosynthesis, DNA replication and repair, and folate coenzyme biosynthesis (44).

Cell wall biosynthesis is disrupted by the β-lactam antibiotics and a class of glycopeptides known as the vancomycins (20, 44). The cell wall of bacteria are of two variations; Gram-positive and Gram-negative (20, 30, 44). Both of these are composed of a peptidoglycan layer in the cell wall structure (33). In the Gram-positive cell wall, however, there is much more peptidoglycan typically in layers, therefore forming a significantly thicker cell wall (44). Built within the thick peptidoglycan layer of the Gram-positive wall are carbohydrates and proteins which serve a variety of purposes including protection against host cell immune responses, composing ligands for attachment to surfaces, and adhesion proteins for the transformation from single cell forms to biofilm communities (44). The Gram-negative cell wall has two components. The cell wall is made of a thinner peptidoglycan layer and an outer membrane layer (44). The outer membrane layer consists of similar proteins and carbohydrates found in the thick peptidoglycan layer of the Gram-positive wall however these are accompanied by porins and are embedded in a phospholipid inner leaflet and a lipid A/O-antigen outer leaflet (44). The Gram-negative wall's complexity functions in giving the bacteria that possess it a lower permeability to outside substances including antibiotics (33, 44).

The peptidoglycan layers found in both types of walls have two types of strands, an orthogonalglycan and peptide that involve a cross link mechanism to join the two (44). This cross link mechanism incorporates the work of two enzymes, a transglycosylase and a transpeptidase (9, 44). These enzymes and precursor mechanisms or proteins are largely the

target of antibiotics that affect cell wall synthesis. β-lactam antibiotics target transpeptidation by blocking the mechanism (44). This type of antibiotic includes penicillins, cephalosporins, carbapenems, monobactams, and clavams (9,44). When administered, the antibiotics work by targeting a variety of penicillin binding proteins called PBPs (44). The PBPs are so named because they have an affinity for binding to the β-lactam ring of β-lactams of which a type is penicillin. In binding to the β-lactam ring of β-lactams, the transglycosylase and transpeptidase enzymes are halted. This causes irregularities and instabilities in the cell wall eventually leading to cell wall degradation or lysis. The outcome is inevitably cell death. Glycopeptides, like vancomycin, function by targeting substrates needed for transpeptidaton (45). In essence, the main role of glycopeptides is to "sequester" the binding end of a peptide strand needed prior to the cross link mechanism in building a cell wall (45). Without the available strand for binding, the cell wall is left vulnerable to anomalies leading again to eventual cell death.

The disruption of protein biosynthesis targets mainly ribosomes which is an important protein in bacteria and is made up of a 30S and a 50S subunit. Aminoglycosides and tetracyclines target the 30S subunit, while macrolides target the 50S subunit of a ribosome (20, 44). A ribosome is an RNA/protein complex responsible for assembling a myriad of proteins from 20 amino acids encoded off of a strand of ribonucleic acid (RNA) through a process called translation (44). Translation begins when the ribosome subunits bind together to translate a strand of RNA known as messenger RNA (mRNA) (44). Translation of proteins is essential to a plethora of processes that allow the cell to survive. This process involves many steps that must be mentioned before discussing how exactly translation is targeted.

During translation, the 30S subunit binds an mRNA strand and is guided into place by the Shine-Dalgarno sequence (30). Once this happens, the initiating t-RNA then binds the 50S

subunit and combines with the growing complex. This makes a whole functional ribosome which can then translate the rest of the codons encoded in the mRNA via transfer RNAs (t-RNA) (30, 44). These t-RNA molecules occupy the ribosome in two distinct sites known as the A-site and the P-site (30, 44). The A-site is the active site upon which t-RNAs enter the ribosome while the P-site is where the t-RNAs exit the ribosome (30, 44). Each t-RNA binds to its own particular amino acid outside of the ribosome (30, 44). Once bound to the amino acid, the t-RNA becomes aminoacylated (30, 44). The A-site is also the area in which the amino acid chain begins to form (30, 44). With each successive entrance of aminoacylated t-RNA into the A-site an enzyme known as peptidyltransferase transfers the elongating amino acid chain to the next aminoacylated t-RNA molecule entering the ribosomal complex (30, 44). As this happens, the deaminoacylated t-RNA then moves to the P-site where it becomes ready to leave the ribosomal complex (30, 44). As such, the A and P site are constantly occupied as elongation continues (30, 44). The ribosome will then eventually encounter a code on the mRNA signaling the termination of the elongating amino acid chain. Upon termination, the 50S and 30S subunits become disassociated (30, 44).

Erythromycins, which are a type of macrolide, function by blocking the entrance of incoming aminoacylated t-RNAs into the A-site of a 50S ribosomal subunit by stalling an occupying aminoacylated t-RNA, prior to peptide transfer, at the P-site before the molecule can exit the ribosomal complex (44). This causes the early release of the aminoacylated t-RNA before peptidyltransferases can carry out their purpose (20, 44). Erythromycins also function by causing the premature release of the 50S subunit before the ribosomal complex is ever in reach of the designated stop codon on the mRNA (44). Tetracylines function similarly to Erythromycins in that they restrict access to the A-site. It does so in a different way; however, a

tetracycline will bind to a groove near the A-site on the 30S ribosomal unit which causes improper binding of an aminoacylated t-RNA to the A-site (44). This delays peptidyltransferase activity as the ribosome recognizes difficulty in movement of the aminoacylated t-RNA onto the P-site (44). Aminoglycosides such as kanamycin and gentamycin, function by binding to the 30S subunit at the A-site during initiation so that the ribosome cannot move past initiation stages (44). Aminoglycosides can also bind to other regions on the 30S subunit and cause changes to the ribosomal molecule that lead it to "misread" the mRNA (44). The action of the above mentioned antibiotic classes causes disruption in the formation of essential proteins needed for the cell to live.

DNA replication and repair are targeted by the quinolones (9, 20, 44). DNA naturally exists in a supercoiled double helix that cannot be replicated as is. Many different types of molecules called topoisomerases are needed to first uncoil then weaken and break the bonds that hold the double helix together (30). Quinolines can target any of these topoisomerases. One type of topoisomerase is DNA gyrase which functions in temporarily breaking the bonds that hold a double helix DNA strand together and separating the DNA into two single strands (30). Only after the DNA double helix is transformed into single strands can the DNA then be replicated (30). Quinolines, such as the one that targets DNA gyrase, function specifically by binding to the newly separated single stranded DNA (20, 44). DNA repair mechanisms and molecules are then deployed to try to repair the anomaly. As the molecules discover that repair is not possible, the bacterial cell essentially is killed by a type of programmed cell death (44).

Sulfa drugs and trimethoprims target the folate coenzyme biosynthetic pathway which is critical in creating components for DNA synthesis (20, 44). Sulfamethoxazole-trimethoprims block the action of two enzymes called dihydropteroate synthase and dihydrofolate reductase

7

(44). Dihydropteroate synthase is crucial in the biosynthetic pathway to folate while dihydrofolate reductase provides a precursor needed in DNA biosynthesis (44). That precursor is tetrahydrofolate which comes from the reduction of dihydrofolate by dihydrofolate reductase (44). Sulfamethoxazole functions in inhibiting dihydropteroate synthase so that folate production declines (44). Trimethoprims are found in combination with sulfamethoxazole because they, in turn, function by binding to dihydrofolate so that it can never be reduced properly by dihydrofolate reductase into tetrahydrofolate (44). The decline of folate and its precursor molecule tetrahydrofolate then causes the cell to become unable to synthesize DNA. Therefore, the bacteria and the infection it may be causing are eradicated.

Resistance to Antibiotics

Antibiotic resistance has had its origins from two distinct paths, natural and acquired (9). Although it is easy to think of antibiotics as a man-made chemical, they were in fact first isolated from microorganisms (20). The exact origins of antibiotics from microorganisms are unclear. There are several theories that try to explain the occurrence. The most noted theory is that antibiotics were produced by microbes as a response to environmental competition (9, 20, 44). Another is that antibiotics are actually products of signaling or nutrient sequestration that just happened to have eradicating properties to foreign microbes (44). Just as antibiotics occur naturally, a response such as resistance occurs naturally as well. In fact, it has been historically noted that the microorganisms which produce antibiotics have genes that prevent toxicity from their own antibiotic (9, 20, 44). In other words, they have resistance to the antibiotic themselves.

These genes are found mainly in genetic elements that are considered mobile (33). These mobile genetic elements are found in the form of insertion sequences, integrons, transposons, and

plasmids (13). The mobilization of these genes is made possible by horizontal gene transfer processes such as conjugation and through infection by a bacterial virus also known as bacteriophage or phage (33).

With the discovery of manufactured antibiotics, a second type of antibiotic resistance emerged, known as acquired resistance (9). Acquired resistance, is generated from the influence of antibiotics (9). When exposed to antibiotics, microorganisms face selective pressures which can cause mutations to the genes of the exposed microorganism (9). When a mutation occurs that allows the microbe to survive in the presence of antibiotics, it gives rise to a microbe better equipped to handle antibiotics. These mutated genes are also transferred via horizontal gene transfer adding to a large repertoire of already existing resistance genes (33).

These genes provide the bacteria with mechanisms to counteract the antibiotic effects. For example, bacteria can counteract β-lactams by producing an enzyme known as a β-lactamase (44). In the presence of a β-lactam antibiotic, a β-lactamase will arrest the effect of antibiotics by hydrolyzing the β-lactam ring before it can reach their target, PBPs (44). Therefore, PBPs are free and do not bind to penicillins in which β-lactams are included (44).

Glycoproteins such as vancomycin can be counteracted in a very elaborate way by a bacterial cell. There are two possible enzymes that can be produced. One is a D-alanine:D-alanine ligase while the other is a D-specific α-keto acid reductase (7). These two carry out two similar processes, both of them will change the chemical nature of the peptide strands found in a building cellular wall and alter them in a way where glycoproteins such as vancomycins cannot recognize the peptide (7). They do not bind, allowing the cross link mechanisms to continue. This leaves the cell wall intact in the presence of vancomycin.

Resistance to tetracyclines is also impressive. Bacteria can offset tetracycline effects in three ways. The three are by restricting the access of the ribosome to tetracycline, changing the structure of the ribosome so that tetracycline does not bind, and finally producing enzymes that deactivate tetracycline (36, 38, 41). In Gram-negative bacteria, porins become altered to limit the entry of tetracycline into the cell (36, 38, 41). In this manner, access to the ribosome is restricted. There are also tetracycline efflux channels. These channels are found in the internal plasma membrane and serve as a detector of tetracycline compounds (36, 38, 41). These compounds are pumped out of the cell before they reach the ribosomal target (36, 38, 41). The bacteria can also produce ribosomal protection proteins. These proteins are produced to bind to the ribosome so that a conformational change occurs in the ribosome (38, 41). This change in conformation leaves the ribosome active while no longer available for tetracycline binding (36, 38, 41). Finally, modification of tetracycline via enzymatic activity is still under investigation. It is likely that they are all originating from an NADPH-requiring oxidoreductase (18, 36, 41).

Resistance to macrolides such as erythromycin actually comes in a variety of ways. One way is the methylation of the 50S subunit of a ribosome via a methylase to decrease the affinity of a macrolide to its binding site (15). Another way is the modification of the macrolide itself by enzymes known as esterases (44). Finally, bacteria resistant to macrolides also harbor resistance in the form of efflux pumps that will channel the antibiotic out of the bacteria even after it has entered the cell (15).

Aminoglycosides like kanamycin and gentamycin are neutralized by two mechanisms. The bacteria will either have a decreased uptake and accumulation of the aminoglycoside or it will be modified by enzymes to become inactive in the bacterial cell (29). The mechanisms for decreased uptake are still unknown, while the decrease in accumulation of aminoglycoside is

largely due to efflux mechanisms similar to the ones seen in tetracycline resistance (29). Efflux

mechanisms will actively bind to aminoglycosides and remove them from within the cell (29).

This phenomenon is currently being investigated. Currently, there are three types of modification

enzymes known which are N-acetyltransferases, *O*-nucleotidyltransferases, and *O*-

phosphotransferases (29). These enzymes will all modify the aminoglycosides to the point where

they can no longer bind to the 30S ribosomal subunit (29).

The effect of sulfa drugs and trimethoprims, such as trimethoprim-sulfamethoxazole, are

offset by modifications to the two enzymes originally targeted by the two antibiotics. It is known

that dihydrofolate reductase and dihydropteroate synthase are modified so that either of the two

antibiotics do not recognize these enzymes and can thus no longer inhibit their function in the

folate coenzyme pathway (25). In the case of dihydrofolate reductase, the enzyme becomes

modified so that it is not dependent on its usual substrate, the modified dihydrofolate reductase

instead uses thymine found outside of the cell (25). In this manner, even as trimethoprim

antibiotics are present, the folate pathway is not inhibited. Resistance to sulfadrugs, such as

sulfamethoxazole usually found in compound with trimethoprim, originates from the

modification of several residues found on the enzyme dihydropteroate synthase (25). As the

enzyme is modified, the usual target is no longer present for the sulfa drug to exert its inhibiting

properties (25).

Currently, measurements to analyze resistance to antibiotics are available through

Clinical and Laboratory Standards Institute (CLSI). This study employed methods found in the

Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational

Supplement as well as from Performance Standards for Antimicrobial Susceptibility Testing;

Seventeenth Informational Supplement. These supplements provide recommended forms of

testing antibiotic susceptibility in many microorganisms. In this study, disk diffusion with zone diameter and minimal inhibitory concentration (MIC) breakpoint interpretive standards were used. The CLSI protocol provides instructions which detail the proper growth and antibiotic susceptibility testing of *Staphylococcus spp*. organisms. Antibiotic resistance is measured via interpretation of a zone diameter recorded in millimeters (mm) (11, 12). For example, when using a kanamycin antibiotic disk of 30 μg to test a staphylococcal sample, the extent of organism's growth nearest to the disk is measured to the nearest millimeter (11, 12). The organism will likely grow by forming a circle around the antibiotic disk and the millimeter measurements are interpreted as follows using kanamycin as an example: sensitive for growth greater than or equal to 15 mm, intermediate for growth between 13-14 mm, and resistant if growth is less than or equal to 12 mm from the disk (11, 12).

In organisms such as *Staphylococcus spp*. antibiotic resistance has historically been linked to a genetic element known as *mecA*. This is a genetic element that is accompanied by one of eight possible genes that detail antibiotic resistance in the organism. This accompanying element is a Staphylococcal Cassette Chromosome *me* more simply termed SCC*mec.* These two genetic elements are discussed further in the section called *mecA* and SCC*mec* elements.

Lower Rio Grande Valley

The Lower Rio Grande Valley is an area found in the southern most point of Texas. It is a border region to Mexico and is comprised of four counties (28). These counties are Starr, Hidalgo, Willacy, and Cameron County (28). According to the Texas State Data Center, in 2008 the Lower Rio Grande Valley (LRGV) was inhabited by 1,138,872 residents (37). The U.S.

Census Bureau in 2008 estimated that each of the Counties of the LRGV was populated by an 85-97% Hispanic demographic (37).

Residents of this area can earn an average per capita income of $5559 to $8899 (34). This is in stark contrast to the average personal annual income of $30,511 for the nation (28). The LRGV has historically and consistently been ranked among highest in unemployment rates, lowest in income, and highest in poverty rates across the United States (28). Of consideration is the fact that in addition to being one of the poorest areas nationally, it is one the fastest growing as well (28). These low income circumstances are an influencing factor when deciding methods of health care for an individual.

Many inhabitants of the LRGV and other border communities understand that healthcare in Texas is not an ideal place to look for treatment for any illness or disease, as they cannot afford the medical treatment from local clinics and, much less, hospitals. Within reach for these resident, however, is alternative treatment from across the border into Mexico where healthcare is much more affordable and the culture is very familiar (21).

Within the LRGV, there are approximately six border-crossing areas that are accessible to residents. Until very recently, there were no consistent guidelines or regulations in place for crossing medications such as antibiotics thus, the practice was common and frequent (27, 40). The inhabitants, aside from receiving more affordable healthcare and treatment across the border, also realized that cutting a doctor visit altogether was even more affordable (27). In fact, it is of common practice to self-treat or self-medicate in these types of communities and the practice is often tied into cultural influence as well (27). Typically, a person who has suffered an infection will likely seek cross-border treatment with a physician. However, upon the recurrence of similar

symptoms accompanying the original infection, the individual will then bypass a physician consultation and purchase the same type of antibiotic that was prescribed for the original infection (27). Also not uncommon in practice, is the use of antibiotics as a form of treatment for illnesses such as a cold that are often viral in nature (3).

In this study, the University of Texas-Pan American was chosen as a reference site from which to obtain community associated staphylococci (CA-S) as well as environmentally associated staphylococci (E-S). This site was chosen because the university is comprised of students inhabiting the four counties of the Lower Rio Grande Valley (31). The university therefore, represents the staphylococci found within the LRGV as these individuals are also residents near the U.S./Mexico border.

In an interview conducted recently with the Student Health Services (SHS) at The University of Texas-Pan American, it was mentioned that approximately two new staphylococci skin infections are treated per week (42). During the course of treating staphylococci related infections in the general student population, when tested for identification, it was typically *S. aureus* that was the source of the infection (42). These infections are typically treated with gentamycin and bactrim, which is a type of trimethoprim-sulfamethoxazole, for a course of ten days (42). The location of infection in the patients is typically in areas that can be left behind by touch and via contact with surfaces (42).

SHS also routinely asks patients about self-treatment though they do not document these responses (42). Based on recall however, it is typical that patients will self-treat with penicillin and amoxicillin (42). SHS also elaborated that despite regulations and recent border violence, their patients report that antibiotics are still fairly accessible (42).

mecA and SCC*mec* elements

Methicillin-Resistant *Staphyloccus aureus* (MRSA) was discovered over 45 years ago as the first organism having resistance to a type of synthetically made antibiotic which included penicillin as well as all β-lactams (17). At the time, MRSA was only present in hospitalized patients and dubbed a hospital acquired infection, however, that soon changed (17). MRSA was later seen in community acquired infections (17). The source of infection was neither directly nor indirectly acquired from hospital settings (17). Studies focused on the source of the antibiotic resistance and ultimately the *mecA* gene was discovered.

The *mecA* gene is found on the chromosome of *S. aureus* and promotes resistance to β-lactams through the production of a penicillin binding protein called PBP2a (23). PBP2a is a transpeptidase enzyme that cross-links peptidoglycans on the cell wall of the microorganism (23). This enzyme is only produced in the presence of β-lactams which allows the organism to continue the cross link mechanisms needed for peptidoglycan formation because it has a very low affinity to β-lactam antibiotics (23). Upstream of the *mecA* gene, are additional regulator genes called *mecR1* and *mecI,* which combined form the *mec* gene comple*x* (26). It has been noted that these regulator genes have opposed functions with *mecR1* being the inducer of the *mecA* gene while *mecI* is a strong repressor (26). There are four types of *mec* complexes divided into Class A, Class B, Class C, and Class D *mec* complexes (10).

Also of importance is the *ccr* gene complex (10). The *ccr* gene complex functions in encoding recombinases to help in the movement of the element (23). There are five types of *ccr* gene complexes. These genes are separated into *ccrA, ccrB*, and *ccrC* categories. There are four allotypes of *ccrAB* genes and only one *ccrC* gene (23). The *mec* gene complex and the *ccr* gene

15

complex are usually found on a larger mobile vector or element known as Staphylococcal

Cassette Chromosome (SCC) (23). United, the two form the SCC*mec* mobile genetic element.

Also found on SCC*mec,* in zones other than the *mec* gene complex and the *ccr* gene complex, are

areas known as J-regions with the J standing for junkyard (23). These J-regions contain

additional genetic information that is sometimes used to detect SCC*mec* types via molecular

testing such as polymerase chain reaction (PCR) (23).

Currently, there are eleven recognized SCC*mec* types known as types I-XI (43). Some of

these SCC*mec* types have historically been divided via association settings. Types I-III are

usually found in hospital or health care associated infections, whereas types IV-V and VII are

found in community associated infections (22, 24). The association for SCC*mec* types VI and

VIII to XI has not yet been clarified. These are composed of a variety of *ccr* gene and *mec* gene

complexes respectively as follows: type I- *ccrA*1 and *B*1 and class B *mec*, type II - *ccrA*2 and *B*2

and class A *mec,* type III - *ccrA*3 and B3 and class A *mec*, type IV - *ccrA*2 and B2 and class B

mec, type V - *ccrC* and class C *mec*, typeVI- *ccrA*4 and *B*4 and class B *mec* (16), type VII-

*ccrC*2 and *ccrC*8 and class C *mec* (24), type VIII- *ccrA* and *B*4 and class A *mec* (8), type IX-

ccrA1 and *ccrB1* and classC2 *mec*, type X- *ccrA1* and *ccrB6* and class C1 *mec*, type XI- *ccrA1*

and *ccrB3* and class E *mec* (44).

Although there are eleven SCC*mec* types, this study explores the most commonly

occurring types I-V, as types VI-VIII have been very recently discovered and have not been

studied to the extent of the original five types. Each of these SCC*mec* types has several

incorporated genes that lend antibiotic resistance. Among the hospital associated SCC*mec* types

is SCC*mec* type I which has resistance mainly to β-lactams, and SCC*mec* type II which has

resistance to erythromycin and spectinomycin (23). Finally, SCC*mec* type III has the broadest

range of multidrug resistance to macrolidelincosamine-streptogramin, cadmium, tetracycline and mercury (23). The two community associated SCC*mec* types IV and V carry mainly β-lactam antibiotic resistance (23).

It is also important to note that community acquired or associated staphylococci have been studied very thoroughly as they are often commensal with humans, however, staphylococci have not been explored independent of human commensalism. An important feature of this study is its interest in staphylococci surviving in the environment. Here, we explore staphylococci surviving outside of the human body on inanimate objects that are humans commonly come into contact with.

Hypothesis

This study focuses on the extent to which SCC*mec* found in staphylococci in the LRGV differ from historic association setting arrangements. We believe that the facilitated accessibility of antibiotics in the LRGV for residents who have habitually self-treated in the past will be denoted in the presence of SCC*mec* types not in accordance with associations of the past, and these will be found in the community as well as the environment. We believe as well, that we will find multidrug resistance harbored in the staphylococci of the LRGV, again due to direct or indirect influence of antibiotic overuse and misuse. We believe that these SCC*mec* types will also be found in species other than the typical pathogenic staphylococcal species, thus signifying the importance of these other species. Finally, we reason that environmentally collected samples will have a significant profiling difference than that of isolates collected from community association providing us with supporting evidence to consider the two as different association settings.

The testing of the above hypotheses was executed through the collection of two staphylococcal libraries of different associations, environmental and community, by methods described in the following section. An antibiotic resistance profile was obtained to reflect antibiotic resistance in the staphylococci of both associations via antibiotic susceptibility testing also described in detail in the following section. The confirmation of the presence of other species of staphylococci carrying SCC*mec* was presented by polymerase chain reaction testing of isolates collected for the presence of *S. aureus*, *S. haemolyticus*, and *S. epidermidis*. These three species are the largest inhabitant type of staphylococci found in the human bacterial flora; therefore, if these are shown not to be the common type of species present among the libraries, it can be inferred that there is a different type or types of staphylococci present and which harbor the SCC*mec* types and antibiotic resistance. SCC*mec* types present were detected via PCR testing of SCC*mec* types I-V, as these have been the recorded abundant type of SCC*mec* types found in staphylococci as well as the most well-known. Finally, the profiling and analysis of the two libraries and combined libraries for all elements present was facilitated by the use of the Statistical Package for the Social Sciences Software program (SPSS).

CHAPTER II

MATERIALS AND METHODS

In this study, two sets of samples were collected, and the staphylococci cultured and stored. The two sets of staphylococci were labeled environmental staphylococci (E-S) and community associated staphylococci (CA-S). A total of 59 isolates were collected for the CA-S library and 137 isolates were collected for the E-S library. After the collection and storage of the above two staphylococcal sample sets, these were submitted to antibiotic sensitivity/susceptibility testing. The collected samples were also prepared for two types of polymerase chain reaction protocols. Multiplex polymerase chain reaction (PCR) was used to identify the presence of three types of staphylococci; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and the presence of the *mecA* gene. SCC*mec* types I-V were determined via PCR as well. The entire above mentioned procedures are presented below in respective sections.

Collection of the Staphylococcal Sample Sets

The community associated set of staphylococcal samples were obtained from student volunteers attending a South Texas university. IRB approval was obtained for this part of the study and researchers were all compliant with Human Subjects training as per IRB specifications. Student volunteers filled out a consent form and were given a sterile, polyester tipped swab packet containing two swabs.

19

Each of the swab packets were labeled to identify sample number and set classification. The students were then instructed to take each of the polyester tipped swabs separately and rotate the cotton swab around their nasal membranes. Thus using both the polyester tipped swabs for each nostril. Upon completing a self- administered nasal swab, the inoculated cotton tipped swabs were then placed back into the original packet and submitted for staphylococcal culture (1).

The environmental staphylococcal samples were collected from common usage areas of high and low traffic at a South Texas university. Single polyester tipped swabs were moistened using sterile saline solution and were then used to swab surfaces in a rotational manner, thus ensuring swab inoculation. These packets were also labeled to identify sample number and set classification. These samples were also placed back into the original packet and submitted for staphylococcal culture (1).

Culture and Storage of Staphylococcal Sample Sets

The CA-S samples were each cultured, following procedures described by Ammons et al. (1), in 5mL enrichment broth made with Iso-Sensitest broth with the following ingredients: 11.7 g of Iso-Sensitest broth and 11.5 g of NaCl with ddH_2O to a total volume of 500 ml. This solution was then autoclaved for 15 mins at 121ºC. The media was then brought to room temperature where 0.5 ml each of 4 mg/ml sterile solution of Cefoxitin, 8 mg/ml sterile solution of Colistin, and 8 mg/ml sterile solution of Aztreonam was added and gently mixed. These were then distributed into sterile culture tubes. The inoculated solution was then incubated for 24 hours at 37°C.

E-S samples were each cultured on MSA plates via rotating the inoculated swab onto the Mannitol Salt Agar (MSA) media. Each plate was then submitted to isolation streaking in an attempt to isolate colonies. The MSA plates were then incubated for 24 hours at 37°C.

After a 24 hour period of growth, both the sample sets were examined for possible staphylococcal morphology. The presence of staphylococci was indicated by the yellowing of the MSA plates demonstrating mannitol fermentation, while the morphology was consistent with a circular white or golden brown colony. When suspected staphylococci were present, a single colony was then transferred onto an additional MSA plate for further purification. Again, the MSA plates were incubated for 24 hours at 37°C. Once the samples underwent this final MSA purification, they were stored in a cryogenic vial containing yeast extract/ tryptone (YT) media supplemented with glycerol for a final concentration of 15% and frozen at -80ºC for later investigation. Samples were logged into the lab's relational database, MicroTracker.

Antibiotic Susceptibility Testing

The stored sample sets were then re-plated on an MSA media plate in preparation for antibiotic susceptibility testing. Antibiotic susceptibility testing was done according to Clinical Laboratory Standard Institute guidelines (11, 12). After a 24 hour incubated growth phase at 37°C, the isolated colonies were transferred to Tryptic Soy Agar (TSA) plates. These were also incubated at 37°C for 24 hours. After the final TSA growing phase, a single colony from the TSA plate was transferred to a tube containing phosphate buffered saline (PBS) at a pH of 7.4, described in preparation for 1 liter of PBS as follows. A mass of 8.0 g of NaCl and 0.2 g of KCl were dissolved in distilled H_2O (ddH_2O). Also added to this solution was 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4. Upon adjustment of the solution's pH, additional ddH_2O was added to adjust

the final volume to 1 liter. This was autoclaved for 15 minutes and stored at room temperature

until ready for use.

The inoculated PBS was vortexed and compared with a 0.5 McFarland Standard solution

tube for proper turbidity. The 0.5 McFarland Standard was prepared as follows: a 0.5-ml aliquot

of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \cdot 2H_2O$) was added to 99.5 ml of 0.18 mol/L H_2SO_4

(1% v/v) and dissolved. The McFarland solution was then transferred to screw cap tubes in

volumes of 3 ml for use. The turbidity of the standard was verified using a spectrophotometer

with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625

nm should be 0.008 to 0.10 for the 0.5 McFarland Standard. Once proper turbidity was

confirmed, a sterile polyester tipped swab was then inserted into the tube with the inoculated

PBS solution and introduced onto the surface of a Mullen Hinton Agar (MHA) plate prepared

according to manufacturer instructions. Inoculation of the plates was performed via continuous

streaks with the swab followed by a turn in the plate at 60°, and a final turn at 60°. The above

mentioned rotation at 60° angles ensured a full plate lawn. Each MHA plate contained 20 mL of

media dispensed with a Pourboy automatic dispenser and were therefore even in depth. An

automatic disk dispenser was used to dispense disks of eight antibiotics onto the surface of all

inoculated MHA plates. The eight antibiotics used for testing were as follows: cefoxitin (30 μg),

erythromycin (15 μg), penicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), vancomycin

(30 μg), tetracycline (30 μg), and trimethroprim-sulfamethoxazole (1.25/23.75 μg). These plates

were then incubated at 37°C and a radius zone of inhibition measurement was taken for all

antibiotics between 16 to 18 hours, except vancomycin which was taken only at 24 hours. The

control strain used along with every testing session was *S. aureus* ATCC 25923. Radial

measurements for the control strain were converted into diameter zones of inhibition

measurements and analyzed for consistency and accuracy according to CLSI documented guidelines (11, 12).

DNA Preparation and PCR Diagnostic Tests

DNA preparation of both the E-S samples and CA-S samples were initiated with a re-plating of the samples from the -80°C cryogenic storage tubes onto TSA media plates. These were then streaked in a four quadrant manner and incubated for growth for 24 hours at 37°C. After the 24 hour growth period, a single colony from each sample was placed into a 0.65 ml microcentrifuge tube along with 100µl of sterilized distilled deionized water (ddH$_2$O). The microcentrifuge tubes were vortexed and then placed onto a hot plate where they were heated for 15 minutes at 100°C to allow for cell rupture. The microcentrifuge tubes were then centrifuged for 2 mintues to form a pellet at the bottom of the tube of all cell remnants. The supernatant solution containing DNA was then used for the PCR protocols or frozen at -20 °C until use.

Each of the samples were then processed through a PCR test which detected the presence of *S. auerus, S. epidermidis, S. haemolyticus* (32), and the presence of the *mecA* gene element (39) with the respective paired forward and reverse primers as described by Pereira et al. and Santos et al.: Nuc1F, Nuc2, SE1, SE2, SH1, SH2, MRSA1 and MRSA2. This reaction ran for 32 cycles with cycle conditions of 10s at 94°C, 10s at 60°C, and 20s at 72°C. The amplified DNA from this PCR was then loaded onto a 2.5% electrophoresis gel containing ethidium bromide at a final concentration of 0.5 µg/ml and run until bands were well separated.

A second test performed on the samples confirmed the presence of SCC*mec* types I-V with the paired forward and reverse primers: beta, alpha3, ccrCF, ccrCR, 1272F1, 1272R1,

5RmecA, and 5R431 (6). This reaction ran for 32 cycles with cycle conditions of 10s at 94ºC, 30s at 56ºC, 60s at 74ºC and a final extension for 4 minutes at 72ºC.

Analysis via Statistical Package for the Social Sciences Software

The data generated from this study was analyzed through the Statistical Package for the Social Sciences (SPSS) program software. The data was put into the program with the isolate names in the rows while the columns were occupied by the data as follows: the library which the isolate belongs to, the presence of *mecA*, the eight antibiotics tested, the species identified, and the SCC*mec* type identified. The designation for libraries was assigned as 1 or 2. In this case, 1 was given the value of CA-S while 2 were given the value of E-S. The *mecA* data was assigned the numbers 0 and 1 where 0 had a value of being *mecA* negative while 1 had a value of being *mecA* positive. The antibiotic data was given values between 1 and 3. Here, 1 was given the value of sensitive, 2 were intermediate, and 3 were resistant. The data for species identification was assigned the numbers 0 to 3 where 0 meant none of the species were identified via PCR, 1 meant the isolate was identified as *S. epidermidis*, 2 meant the isolate was identified as *S. haemolyticus*, and 3 meant the isolate was identified as S. *aureus*. Finally, the SCC*mec* data was given the values 0 to 10 where 0 meant none of the SCC*mec* types were identified via PCR, 1 meant the isolate was identified as SCC*mec* type I, 2 meant the isolate was identified as SCC*mec* type II, 3 meant the isolate was identified as SCC*mec* type III, 4 meant the isolate was identified as SCC*mec* type IV, 5 meant the isolate was identified as SCC*mec* type V, 6 meant the isolate was identified as Novel Combination (NC) Type A, 7 meant the isolate was identified as NC Type B, 8 meant the isolate was identified as NC Type C, 9 meant the isolate was identified as NC Type D, and 10 meant the isolate was identified as NC Type E.

The data was then analyzed using two different statistical analysis features in the program. The first statistical analysis that was used was the Kruskal-Wallis test under the nonparametric tests selection. This test was used to compare all of the data presented in columns among both of the libraries. The second analysis used in this study was the cross tabulation feature found under the descriptive statistics selection. This feature was used to analyze the species identified data with respect to the antibiotic resistance data. Also analyzed through this feature was the species identified data with respect to the SCC*mec* type data, and the SCC*mec* type data with respect to the antibiotic resistance data.

CHAPTER III

RESULTS

Species Identified and *mecA* in Libraries

According to results from the polymerase chain reaction tests identifying the presence of *mecA* and three species, the data shows that among the CA-S library, 66.1% were *mecA* positive. Among the E-S library, 21.9 % were *mecA* positive. This data along with species identification can be found in Table 1 below. In reference to species identification, within the CA-S library 45.8% were not identified be the PCR primers, 23.7% were identified as *S. epidermidis*, 23.7% were identified as *S. haemolyticus*, and 6.8% were identified as *S. aureus* (Table 1). In the E-S library 73.7% were not identified by the PCR primers, 2.2% were identified as *S. epidermidis*, 20.4% were identified as *S. haemolyticus*, and 3.6% were identified as *S. aureus* (Table 1).

Table 1. Species Composition and *mecA* Among Libraries

		Results	Frequency	Percent
CA-S	*mecA*	Negative	20	33.9
		Positive	39	66.1
		Total	59	100
	Species	None	27	45.8
		S. epidermidis	14	23.7
		S. haemolyticus	14	23.7
		S. aureus	4	6.8
		Total	59	100
E-S	*mecA*	Negative	107	78.1
		Positive	30	21.9
		Total	137	100
	Species	None	101	73.7
		S. epidermidis	3	2.2
		S. haemolyticus	28	20.4
		S. aureus	5	3.6
		Total	137	100

Antibiotic Resistance Profiles in Libraries

According to results from the antibiotic susceptibility testing for the occurrence of resistance in percentage of the total among the eight antibiotics tested are as follows for the CA-S library: 61% were resistant to cefoxitin, 54.2% were resistant to erythromycin, 18.6% were resistant to gentamycin, 22% were resistant to kanamycin, 76.3 % were resistant to penicillin, 10.2% were resistant to tetracycline, 5.1% were resistant to trimethoprim-sulfamethoxazole, and 1.7% were resistant to vancomycin. The antibiotic susceptibility testing for the occurrence of resistance in percentage of the total among the eight antibiotics tested are as follows for the E-S library: 22.6% resistant to cefoxitin, 32.1% resistant to erythromycin, 4.4% resistant to gentamycin, 8.8% resistant to kanamycin, 43.8% resistant to penicillin, 7.3% resistant to tetracycline, 6.6 % resistant to trimethoprim-sulfamethoxazole, and 1.5% resistant to

vancomycin. The above mentioned results can be found along with occurrence of sensitivity and intermediacy in Table 2 below.

Table 2. Antibiotic Susceptibility Among Libraries

Frequency	Percent	E-S	Antibiotic	CA-S	Frequency	Percent
106	77.4	Sensitive	Cefoxitin	Sensitive	23	39
31	22.6	Resistant		Resistant	36	61
77	56.2	Sensitive	Erythromycin	Sensitive	20	33.9
16	11.7	Intermediate		Intermediate	7	11.9
44	32.1	Resistant		Resistant	32	54.2
131	95.6	Sensitive	Gentamycin	Sensitive	48	81.4
6	4.4	Resistant		Resistant	11	18.6
118	86.1	Sensitive	Kanamycin	Sensitive	45	76.3
7	5.1	Intermediate		Intermediate	1	1.7
12	8.8	Resistant		Resistant	13	22
77	56.2	Sensitive	Penicillin	Sensitive	14	23.7
60	43.8	Resistant		Resistant	45	76.3
123	89.8	Sensitive	Tetracycline	Sensitive	52	88.1
4	2.9	Intermediate		Intermediate	1	1.7
10	7.3	Resistant		Resistant	6	10.2
128	93.4	Sensitive	Trimethoprim-sulfamethoxazole	Sensitive	55	93.2
0	0	Intermediate		Intermediate	1	1.7
9	6.6	Resistant		Resistant	3	5.1
128	93.4	Sensitive	Vancomycin	Sensitive	58	98.3
9	6.6	Resistant		Resistant	1	1.7

Novel PCR SCC*mec* Band Combinations found in Libraries

Of noteworthiness is the emergence of novel band combinations when the multiplex PCR test was performed among both libraries for SCC*mec* type identification. The multiplex PCR test used for this study employed the presence of four bands in unique combinations to identify the presence of SCC*mec* types I-V. The original identifying combinations according to band markers are as follows: type I- band 3, type II- band 1, type III- band 2, type IV- band 1 and 3, and type V- band 2 and 4 (Figure 1). However, several novel combinations were noted as the libraries

were tested. These novel combinations were deemed Novel Combination (NC) Type A through

E. Their band combinations were as follows: NC Type A- band 3, 2, and 1, NC Type B- band 3

and 2, NC Type C- band 4, 3, and 2, NC Type D- band 4, 2, and 1, and NC Type E- band 4 and 1
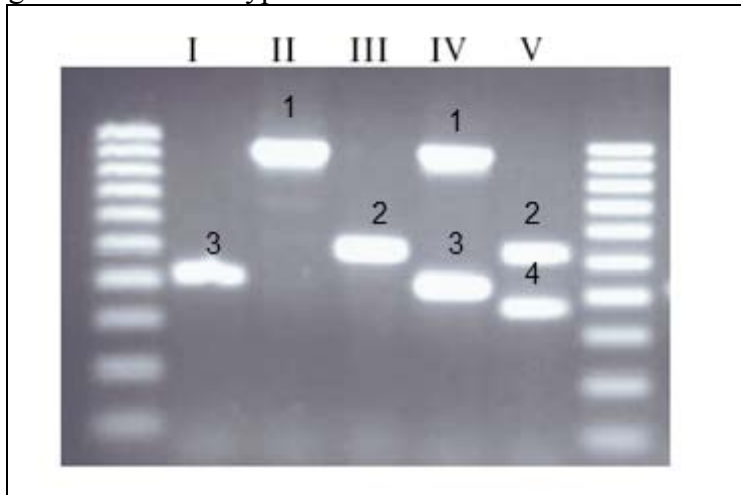
(Figure 2).

Figure 1. SCC*mec* Types I-V

Figure taken from Boye et al. 2007, demonstrates band combinations present in the five SCC*mec*
types denoted by the numbers 1-4.

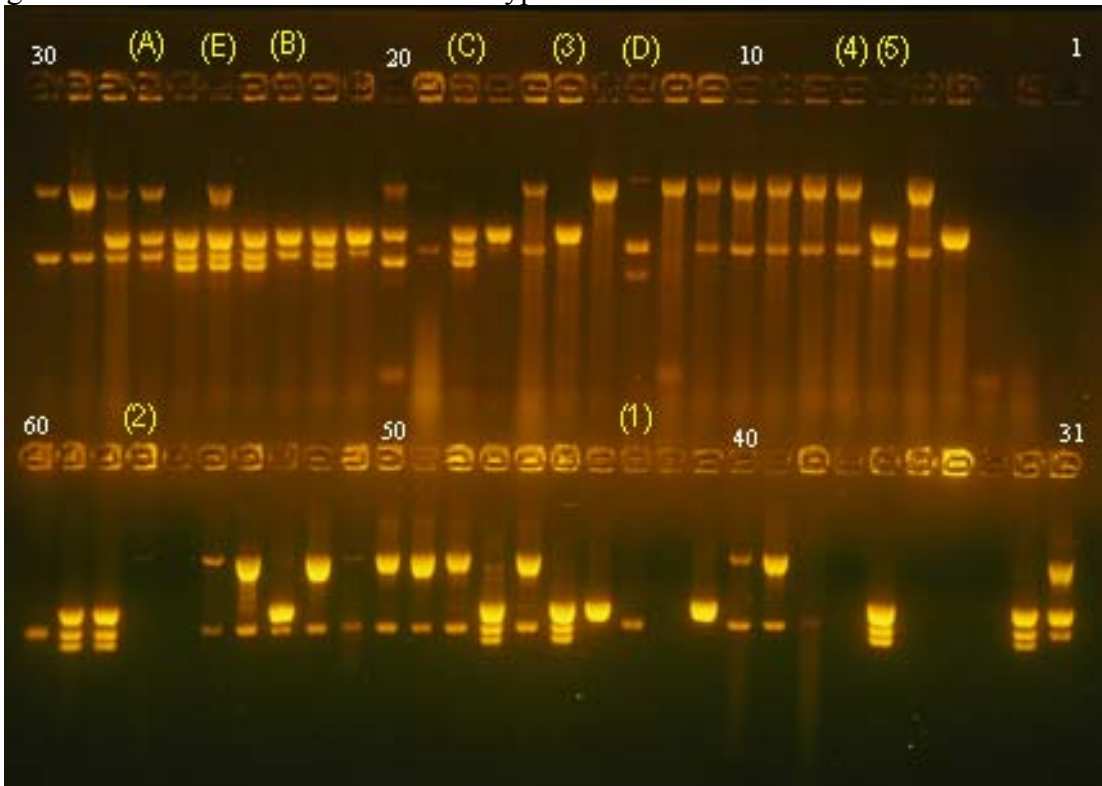Figure 2. Novel Combination SCC*mec* Types Identified

Figure demonstrates NC Types A-E denoted by the yellow letters A-E in accompanied by SCC*mec* types I-V denoted by the yellow numbers 1-5 in parenthesis.

SCC*mec* and Novel Combination Types in Libraries

According to the multiplex PCR test used to identify the presence of SCC*mec* types I-V and NC type emergence the results for the CA-S library were as follows: 22% of the isolates had none of the SCC*mec* types identifiable by the multiplex PCR, 5.1% were type I, 1.7 % were type II, 11.9% were type III, 20.3% were type IV , 6.8% were NC Type A, 5.1% were NC Type B, 23.7% were NC Type C, 1.7% were NC Type D, and 1.7% were NC Type E. The results for the E-S library were as follows: 70.8% of the isolates had none of the SCC*mec* types identifiable by the multiplex PCR, 8.0% were type I, 5.1% were type III, 3.6% were type IV, 0.7% were type V,

1.5% were NC Type A, 3.6% were NC Type B, and 6.6% were NC Type C. These results can be found on Table 3 below.

Table 3. SCC*mec* Types Among Libraries

Library	SCC*mec* Types	Frequency	Percent
CA-S	None	13	22
	Type 1	3	5.1
	Type 2	1	1.7
	Type 3	7	11.9
	Type 4	12	20.3
	NC Type A	4	6.8
	NC Type B	3	5.1
	NC Type C	14	23.7
	NC Type D	1	1.7
	NC Type E	1	1.7
	Total	59	100
E-S	None	97	70.8
	Type 1	11	8
	Type 3	7	5.1
	Type 4	5	3.6
	Type 5	1	0.7
	NC Type A	2	1.5
	NC Type B	5	3.6
	NC Type C	9	6.6
	Total	137	100

Comparison of Both Libraries

A Kruskal-Wallis test of both libraries via SPSS showed significant difference among both libraries for the data presented for *mecA* ($p \leq 0.001$), SCC*mec* ($p \leq 0.001$), species ($p = 0.002$), erythromycin ($p = 0.002$), gentamycin ($p = 0.001$), cefoxitin ($p \leq 0.001$), and penicillin ($p \leq 0.001$) Table 4. There was no significant difference among the data presented for kanamycin (p

= 0.064), tetracycline (p = 0.707), trimethoprim-sulfamethoxazole (p = 0.980), and vancomycin

(p = 0.902) Table 4.

Table 4. Kruskal Wallis Test of all Elements Tested

	mecA	SCC*mec*	Species	E	Ge	Ce	K	P	Te	SXT	Va
Chi-square	35.146	44.009	9.507	9.267	10.539	26.876	3.420	17.398	.142	.001	.015
df	1	1	1	1	1	1	1	1	1	1	1
Asymp. Sig.	.000	.000	.002	.002	.001	.000	.064	.000	.707	.980	.902

For the table above, E=Erythromycin, Ge= Gentamycin, Ce=Cefoxitin, K= Kanamycin, P= Penicillin, Te=
Tetracycline, SXT= Trimethoprim-sulfamethoxazole, and Va= Vancomycin.

Exploration within Libraries

The SPSS software was also used to generate a cross tabulation analysis, based on counts,

among species identified in the study within both combined libraries and the presence of *mecA*

and the SCC*mec* types (Table 5), among SCC*mec* types and *mecA* presence (Table 6), among the

species identified in the study within both combined libraries and the antibiotic resistance

profiles (Table 7), and finally the SCC*mec* types along with the presence of *mecA* identified in

the study within both combined libraries and the antibiotic resistance profiles (Table 8). These

analyses were conducted in an attempt to distinguish associations across species, SCC*mec* types,

mecA presence, and antibiotic resistance profiles.

Table 5. Species VS SCC*mec* Type and *mecA* Among Libraries

Species Present	*mecA*		SCC*mec* Type										
	(+)	(-)	None	T 1	T 2	T 3	T 4	T 5	NC T A	NC T B	NC T C	NC T D	NC T E
None	20	108	101	4	1	12	6	0	1	2	0	0	1
S. epi	16	1	1	1	0	1	8	0	4	1	0	1	0
S. haemo	29	13	4	8	0	1	1	1	1	5	21	0	0
S. aureus	4	5	4	1	0	0	2	0	0	0	2	0	0
Total	69	127	110	14	1	14	17	1	6	8	23	1	1

For the table above, *S.epi= S. epidermidis, S. haemo= S. haemolyticus*, (+)= positive, (-)= negative, T 1= SCC*mec* Type I, T 2= SCC*mec* Type II, T 3= SCC*mec* Type III, T 4= SCCmec Type IV, T 5= SCC*mec* Type V, NC T A= Novel Combination Type A, NC T B= Novel Combination Type B, NC T C= Novel Combination Type C, NC T D= Novel Combination Type D, NC T E= Novel Combination Type E.

Table 6. SCC*mec* VS *mecA* Among Libraries

SCC*mec* Type	*mecA*		
	Negative	Positive	Total
None	98	12	110
T 1	11	3	14
T 2	1	0	1
T 3	11	3	14
T 4	1	16	17
T 5	0	1	1
NC T A	1	5	6
NC T B	4	4	8
NC T C	0	23	23
NC T D	0	1	1
NC T E	0	1	1
Total	127	69	196

For the table above, T 1= SCC*mec* Type I, T 2= SCC*mec* Type II, T 3= SCC*mec* Type III, T 4= SCC*mec* Type IV, T 5= SCC*mec* Type V, NC T A= Novel Combination Type A, NC T B= Novel Combination Type B, NC T C= Novel Combination Type C, NC T D= Novel Combination Type D, NC T E= Novel Combination Type E.

Table 7. Species VS Antibiotic Resistance Profiles Among Libraries

Species Present	Resistance by Antibiotic Type							
	Ce	E	Ge	K	P	Te	SXT	Va
None	32	42	5	8	57	5	8	2
S. epi	13	11	3	4	13	3	1	0
S. haemo	19	19	9	13	26	8	2	1
S. aureus	3	4	0	0	9	0	1	0
Total	67	76	17	25	105	16	12	3

For the table above*, S.epi= S. epidermidis, S. haemo= S. haemolyticus*, Ce=Cefoxitin, E=Erythromycin, Ge= Gentamycin, , K= Kanamycin, P= Penicillin, Te= Tetracycline, SXT= Trimethoprim-sulfamethoxazole, and Va= Vancomycin.

Table 8. SCC*mec* Types and *mecA* VS Antibiotic Resistance Profiles Among Libraries

mecA & SCC*mec* Type	Resistance by Antibiotic Type								
	Ce	E	Ge	K	P	Te	SXT	Va	Total
mecA (-)	17	37	6	9	45	6	6	2	128
mecA (+)	50	39	11	16	60	10	6	1	193
None	24	31	5	6	47	5	8	2	126
T 1	2	8	1	4	4	2	1	0	22
T 2	0	0	0	0	0	0	0	0	0
T 3	5	9	1	1	6	0	0	0	22
T 4	12	7	1	2	15	2	0	0	39
T 5	0	1	0	0	1	0	0	0	2
NC T A	3	4	0	0	4	0	0	0	11
NC T B	3	1	1	1	5	0	0	0	11
NC T C	16	14	7	10	21	6	3	1	78
NC T D	1	0	1	1	1	1	0	0	5
NC T E	1	1	0	0	1	0	0	0	3
Total	67	76	17	25	105	16	12	3	321

For the table above*,* Ce=Cefoxitin, E=Erythromycin, Ge= Gentamycin, , K= Kanamycin, P= Penicillin, Te= Tetracycline, SXT= Trimethoprim-sulfamethoxazole, and Va= Vancomycin, (-)= negative, (+)= positive, T 1= SCC*mec* type I, T 2= SCC*mec* type II, T 3= SCC*mec* type III, T 4= SCC*mec* type IV, T 5= SCC*mec* type V, NC T A= Novel Combination Type A, NC T B= Novel Combination Type B, NC T C= Novel Combination Type C, NC T D= Novel Combination Type D, NC T E= Novel Combination Type E.

CHAPTER IV

DISCUSSION

Species Identified and *mecA* in Libraries

According to the species composition determined via PCR, one can gather that there is likely an assortment of *Staphylococcus* species yet to be identified among the CA-S and E-S libraries. Originally, it was expected that the majority of the organisms found in the CA-S library would be *S. aureus* because as mentioned earlier, it is localized in the human nares which is where the samples were obtained. However, as the PCR revealed, 45.8% of the staphylococci were none of the three species detected by the PCR primers and are also likely carrying the *mecA* gene (66.1% *mecA* positive, Table 1). We consider these unidentified species to be a type of staphylococci because of the selective media in which they were originally grown. Regard as well, the fact that these organisms readily carry the *mecA* gene as just mentioned a majority (66.1%) tested positive for the gene. Of the species that could be identified, it seems that an equal portion of *S. epidermidis* and *S. haemolyticus* are residing in the nares of individuals of the LRGV (Table 1). This is interesting because the nasal membranes are not where these particular organisms typically reside according to Crossley et al. (2009).

In the E-S library, an even larger portion (73.7%) was not recognized as any of the three species the PCR was designed to identify. In this library, it was thought that a majority of the organisms would be *S. epidermidis*. This was assumed, since the origin of location for the library was from areas of common human contact via touch. Due to the nature of *S.epidermidis* to be abundant in human skin bacterial flora according to Crossley et al. (2009), it was assumed that these would be the organisms left behind by human touch. Interestingly, fewer of these organisms (21.9%) also carry the *mecA* gene. Of the species that were identified by the PCR in the library, a majority (20.4%) were *S. haemolyticus* and not *S. epidermidis* as expected.

SCC*mec* types in Libraries

According to the results generated from SCC*mec* type identification it is evident that the CA-S collected here, does not follow the traditional association schemes presented in previous literature. As can be seen in Table 3, the CA-S library is made largely of NC Type C followed by SCC*mec* type IV and SCC*mec* type III. Although type IV is historically found in community associated settings, it is followed closely by SCC*mec* type III which is typical of hospital associated settings.

In comparison, the E-S collected, exhibits the presence of SCC*mec* type I, NC Type C, and SCC*mec* type III. Historically, SCC*mec* type I and type III have been found in hospital associated settings. Results such as the ones seen here lead us to question what has happened to the SCC*mec* types found in the LRGV and whether the SCC*mec* type association has become obscured.

Antibiotic Resistance in Libraries

The results generated from the antibiotic susceptibility testing reveals that in the CA-S library penicillin (76.3%), cefoxitin (61%), and erythromycin (54.2%) were the antibiotics leading in antibiotic resistance (Table 2). As for the E-S library, it displayed penicillin (43.8%), erythromycin (32.1%), and cefoxitin (22.6%) as the leading antibiotics in occurrence of resistance (Table 2).

In seeing these results, one can only speculate whether this is an indirect result of antibiotic overuse and misuse. It is also interesting to note that the samples were taken from asymptomatic individuals (CA-S). It leads us to question how these individuals can harbor such resistant organisms in a commensalist manner and the consequence of an upset in nature of the relationship.

Consider also the resistance harbored in the E-S library; these are organisms that are left behind and encountered every day by individuals. As mentioned earlier, there are very finite studies on the resistance harbored in staphylococci living in the environment on inanimate objects. This study reveals that not only can an assortment of species (inferred from species composition data) survive on surfaces, but they also impose a potential danger to those who encounter them based on the resistance found within them.

Establishment of Two Settings

The results generated from the comparison of both libraries (Table 4) via Kruskal Wallis test reveals that there is a significant difference in their *mecA* ($p< 0.001$), SCC*mec* ($p< 0.001$), and species composition ($p= 0.002$). Similarly, the profiles of four out of the eight antibiotics tested are significantly different among the two libraries. It is from these significant differences

that we contend that the two libraries should be recognized as a separate setting of association. The two show significant differences in the majority of the elements tested.

It is also interesting to note the presence of staphylococci in the environment, and how it may establish its difference from that of community associated staphylococci. Staphylococci of environmental association have not been studied to the extent of staphylococci from the human flora. The findings here, would solicit a deeper study into the identity of staphylococci that can inhabit inanimate objects. Until recently, the ability of *S. epidermidis* to adhere to, and form, a substantial biofilm on plastic surfaces was noted by Das et al. (14). Here, we see that it is not *S. epidermidis* that inhabits common surfaces but unidentified staphylococci. Due to the resistance these isolates carry and the SCC*mec* types found among the environmental isolates, it would be sensible to study the species composition further through the partial sequence of the *rpoB* gene, as described by Drancourt and Raoult (2002), and their ability to exchange genetic information without a host organism and without nutrients (19).

Novel Band Combinations

As noted in the SCC*mec* type results (Table 3), novel combinations of bands produced by the PCR were found in consistent patterns which we then deemed Novel Combination Types A-E. The cause of the NC types could be due to perhaps a recombination of SCC*mec* types I-V as originally targeted by the PCR within the genome of the staphylococci. The recombination could be in the form of two types of SCC*mec* or a fragment of an original SCC*mec* type that has been incorporated into a site of a secondary type of SCC*mec*.

For example, NC Type A was identified as having the presence of bands 3 and 2. This could suggest that this particular *Staphylococcus* is carrying a combination of SCC*mec* type I and

III as the two are identified by the PCR as having band 3 and 2 respectively. Similar combinations suggest that the like may be happening with the remaining NC types as illustrated by Figures 1 and 2.

Cross Tabulations and Possible Correlations

A cross tabulation analysis as illustrated in Tables 5-8 reveals several interesting associations. Table 5 shows that the unidentified staphylococci are largely SCC*mec* and *mecA* negative. Of the SCC*mec* types that are present among the unidentified staphylococci, the most prevalent is SCC*mec* Type III. This suggests that while the unidentified species are largely SCC*mec* negative, when present however the prominent SCC*mec* is of hospital association. Among the identified staphylococci species, *S. epidermidis*, *S. haemolyticus,* and *S. aureus* the two most prominent types for each are SCC*mec* type IV and NC Type A, NC Type C and SCC*mec* type I, and NC Type C and SCC*mec* type IV respectively. A closer look into the occurrence of NC Type C reveals that it is present mainly in *S. haemolyticus* and minimally present in *S. aureus*. In terms of this particular element, this could suggest a pattern of movement from one species to another. In addition, *S. haemolyticus* and *S. epidermidis* are mostly *mecA* positive, while *S. aureus* is marginally *mecA* negative.

A cross tabulation analysis was plotted with SCC*mec* identified and the presence of *mecA* in an attempt to identify associations between the two in Table 6. Interestingly, 12 isolates that were SCC*mec* negative were *mecA* positive. This could suggest that other SCC*mec* types not identified by the SCC*mec* PCR test are present in these isolates. In addition, the table revealed that a large portion of isolates identified as having SCC*mec* types I and III were *mecA* negative. Current literature does not suggest that this is typical of the two types, and would therefore

suggest a possible loss of *mecA* or a change in the nucleotide composition of the primer binding areas. A closer look at this revelation is necessary to conclude such an event is happening. The target areas could be sequenced to confirm whether the *mecA* gene has been lost or if there are new polymorphisms in the primer binding areas. Lastly, Table 6 also reveals that, in fact, a great majority of the NC types, except NC Type B, are carrying the *mecA* gene; therefore an association likely exists among *mecA* and the NC types.

According to Table 7, an analysis of species and antibiotic resistance profiles, the species identified and the unidentified revealed a similar profile. It appears that they are all principally resistant to penicillin, erythromycin, and cefoxitin although particularly not in that order. The reported resistance to penicillin and cefoxitin is supported by the presence of the *mecA* gene element in a majority of the species identified as seen in Table 5. For the unidentified species, however, the elevated resistance to penicillin, erythromycin, and cefoxitin is not supported by the *mecA* results as this group is largely *mecA* negative. The antibiotic profile generated also appears to be in contrast to the data reported by the SCC*mec* types among the unidentified species as it was shown that these are largely SCC*mec* negative. We speculate that perhaps the unidentified species are obtaining resistance to the aforementioned antibiotics by a means other than acquiring *mecA* or *SCCmec* elements. It is also possible that these could harbor new variants of the targeted genes to which the primers utilized in this study are not compatible for binding.

In an attempt to correlate the NC types with a particular antibiotic resistance profile, a cross tabulation (Table 8) was run plotting SCC*mec* types and *mecA* presence against antibiotic resistance profiles. Interestingly, it appears that an elevated amount of isolates demonstrated resistance to erythromycin, penicillin, cefoxitin, kanamycin, gentamycin, tetracycline, and trimethoprim-sulfamethoxazole without the presence of the *mecA* gene. This is unexpected, at

least, in terms of the resistance shown to cefoxitin and penicillin as it is the *mecA* gene which bears resistance to the two. Although, no confirmatory association is established, it is noted that a majority of NC Type A appear to have multidrug resistance to the antibiotics penicillin, erythromycin, and cefoxitin. This is expected as it was seen in Table 6 the NC Type A is associated with the presence of the *mecA* gene element. NC Type B potentially has multidrug resistance as well to penicillin and cefoxitin, although this is not substantially supported by the data shown in Table 6. This NC type does not demonstrate a markedly larger association to the *mecA* presence. NC Type C potentially has multidrug resistance as well to penicillin, cefoxitin, erythromycin, and kanamycin. This antibiotic resistance profile is largely supported by the NC type's association to the presence of *mecA* as seen in Table 6. Lastly, due to the low rate of isolates identified with NC Types D and E, an evident antibiotic resistance profile is not visible and therefore none will be suggested for the two.

To conclude, a final tie-in of the information derived in the study leads us to question whether it could be within *S. haemolyticys* or *S. aureus* that a recombination of SCC*mec* is possible. If such an instance is possible, then could this be an insight to the future of antibiotic resistance for the rest of the US as antibiotic use continues? A study conducted by Berglund (2008) suggested that SCC*mec* transfer among the two species could be possible (5). We propose that a similar case is happening here. If horizontal transfer is happening among the two species, we question what other species are a likely match for horizontal transfer and whether we need to focus on other *Staphylococcus* populations for SCC*mec* distribution and possible recombination. The results obtained from this study solicit a closer look into these unidentified staphylococcal species and their novel combination SCC*mec* types to acquire a new genomic target to potentially halt acquisition of antibiotic resistance.

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BIOGRAPHICAL SKETCH

Diana Evelyn Treviño, raised in Mission, Texas, graduated from Mission Veteran's Memorial High School. As a Valley Scholar, she entered South Texas College where she graduated with all possible Honors and received her Associate of Science in 2006.

Upon completing her Associate degree, she enrolled at The University of Texas-Pan American as a Biology major. It was at this institution, where she began her undergraduate research under Dr. Joanne Rampersad. Her research consisted of several fields of interests including electron microscopy, diagnostic testing, molecular biology, and microbiology. During this time, she completed a respected internship at the United States Department of Energy Argonne National Laboratory. She went on to graduate with Magna Cum Laude honors in 2009 with a Bachelor of Science in Biology and a minor in both Chemistry and Psychology.

She continued her academic career at The University of Texas-Pan American and was awarded the University of Texas Medical Branch at Galveston's "Bridges to PhD" Fellowship. During this time she began her career at the United States Department of Agriculture Cattle Fever Tick Research Laboratory where she gained valuable experience in the field. She went on to graduate with a Master of Science in Biology in 2011.

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