PREVALENCE AND CHARACTERIZATION OF STAPHYLOCOCCUS SPECIES,
INCLUDING MRSA, IN THE HOME ENVIRONMENT

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ABSTRACT

The purpose of this study is to assess the prevalence of *Staphylococcus*, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), in a home environment. Staphylococcal species are among the most common bacteria causing joint infections, and emerging methicillin-resistant *Staphylococcus aureus* strains continue to remain a global problem. Staphylococci organisms commonly colonize the skin and nasal nares of healthy individuals, as well as livestock and other animals. There is a growing body of evidence that the environment (surfaces) plays an important role in the transmission of pathogens in the community.

MRSA can be spread from contaminated inanimate objects known as fomites. Many hard surfaces serve as good reservoirs for MRSA, including door knobs and hand rails. In this study, we assessed the prevalence of MRSA and other staphylococcal species in 76 homes across the central Texas area. This study will evaluate various *Staphylococcus* species found on four different areas of a home using a point prevalence design to detect the prevalence of MRSA.

A total of 304 swab samples from 76 different households were collected. Sources of collection included the inside front door handle, kitchen sink handle, most used remote control, and the refrigerator handle. One hundred and eighteen samples showed microbial growth (118/304, 38.8%), and 92 samples tested positive presumptively for *S. aureus* (92/304, 30.3%). Of those 92, four demonstrated oxacillin resistance and three were identified as MRSA (3/92, 3.4%) by displaying mauve colonies on CHROMagar. The findings of this study indicate potential exposure risks from *Staphylococcus* in everyday home environments, especially immunocompromised individuals.
CHAPTER 1

Introduction

1.1 Background

One of the most notorious and deadly antibiotic resistant bacteria strains is methicillin-resistant *Staphylococcus aureus* (MRSA).\(^1\) Methicillin-resistant *Staphylococcus aureus* is a strain of *Staphylococcus aureus* that is resistant to beta-lactam antibiotics. Beta-lactam antibiotics include those such as methicillin, penicillin, oxacillin, and amoxicillin.\(^1\) Over the last decade, the spread of community-acquired MRSA (CA-MRSA) has begun to spread more rapidly due to the increased transmission of resistant strains of MRSA.\(^2\) Due to MRSA being rapidly spread and frequent hand and skin-to-skin contact among individuals in a home, certain groups may be at an increased risk for exposure to MRSA infections.\(^1\)

1.2 Research Motivations

Recently, antibiotics have been increasingly used improperly or unnecessarily leads to an increase in the spread of several strains of antibiotic-resistant bacteria.\(^3\) One of the most notorious and deadly antibiotic resistant bacteria strains is methicillin-resistant *Staphylococcus aureus*\(^2\).

Every year, the United States has an estimated two million people become infected with antibiotic-resistant bacteria, and at least 23,000 victims die as a result.\(^2\) Infections with MRSA have become so prevalent that these infections have surpassed HIV as a leading cause of morbidity and mortality within the United States.\(^4\) One study
has predicted that antibiotic resistance will have an economic impact of $100 trillion dollars and a human impact of 10 million deaths.²

The findings of this study could indicate potential exposure risks from *Staphylococcus* in everyday home environments. The general public could use information from this study, focusing on the understanding of the prevalence of MRSA and other infectious agents, to be more aware of, and engage in, proper hygiene techniques to lower their exposure risk.

### 1.3 Objectives and Scope

Antibiotic-resistant organisms make infections extremely difficult to treat because they are able to survive the drugs that were designed to specifically kill them. Infections involving MRSA continue to take a huge toll on morbidity rates and have remained a leading cause of death within the United States.⁵ Community-acquired MRSA strains are becoming progressively more responsible for an increased number of serious infections in non-hospitalized patients that were previously healthy.⁴

MRSA can be spread from inanimate objects that have become contaminated, known as fomites. One study demonstrated that *S. aureus* can survive on polyester material for up to 56 days and MRSA can remain on the material for up to 40 days.⁴ Many hard surfaces serve as good reservoirs for MRSA, including door knobs and hand rails.⁵ Due to MRSA being able to rapidly spread, and frequent hand and skin-to-skin contact among individuals in a home, certain groups may be at an increased risk for exposure to MRSA infections due to their cultural habits and interactions with one another and with their home environment.⁶
In this study, we assessed the prevalence of *Staphylococcus aureus* and MRSA in 76 homes across the central Texas area. The purpose of this study is to assess the prevalence and characterization of *Staphylococcus* species, including MRSA, in the home environment. While at home, individuals are often relaxing in attire that increases the availability of skin-to-skin contact, such as in athletic shorts and t-shirts. This can increase the potential risk of MRSA exposure for certain individuals in a home environment.
CHAPTER 2

Literature Review

*Staphylococcus aureus* is an incredibly dangerous pathogen that has been responsible for a collection of human infections all across the world.\(^7\) The majority of *Staphylococcus aureus* infections result in moderately severe infections of the respiratory tract or as skin infections.\(^8\) However, *S. aureus* can also cause much more severe and dramatic diseases such as necrotizing fasciitis, which can become life-threatening.\(^7\)

Today, a major problem that physicians face when needing to treat *S. aureus* infections is antibiotic resistance.\(^8\) Penicillin-resistant *S. aureus* strains were first detected against the first antibiotic, penicillin, in 1942.\(^9\) Resistance to penicillin occurred due to the penicillinase activity that is able to cleave the β-lactam ring of penicillin. Soon after, by the 1950’s, strains of *S. aureus* that contained penicillinase were pandemically widespread among hospitals.\(^10\) Today, almost all infectious strains of *S. aureus* are resistant to the antibiotic penicillin.\(^8\)

Scientists soon developed the antibiotic methicillin, which is derived from penicillin, to overcome the problem of penicillin-resistant *S. aureus*.\(^10\) However, just one year later, methicillin-resistant *S. aureus* was soon discovered. *Staphylococcus aureus* epidemics now occur in waves depending on their antibiotic resistance.\(^9\) Even more worrisome is the fact that, for a while, the MRSA epidemic was thought to be limited to hospitalized patients. However, this is no longer the case, as most recent waves of MRSA related epidemics have emerged by CA-MRSA since the late 1990’s.\(^11\)
CA-MRSA lineages have emerged due to the designated widespread clone USA300. In comparison, the common most genotype of hospital-acquired MRSA (HA-MRSA) USA100. Novel MRSA clones continue to keep occurring and are continuously being discovered in hospitals, and now more recently, in the community. Furthermore, MRSA strains are now constantly accumulating increased antibiotic resistance genes that in turn increase the virulence and treatability of these ‘superbugs’.

Since the start of when antibiotic use began to increase, *S. aureus* has continued to rapidly acquire resistance by a variety of genetic mechanisms. The meca gene is spread on the SCCmec genetic element and resistant strains of this gene are responsible for many of the MRSA infection originating in hospitals. MRSA produces a penicillin binding protein 2a that results in resistance to all beta-lactam antibiotics. In CA-MRSA, the production of PVL, secreted proteases, and alpha toxins are suspected as particular virulence determinants.

For persons living in the community that have frequent contact with the healthcare system, nosocomial strain types of MRSA are a frequent cause of infection. The continuous widespread and epidemic that MRSA causes has shown that it is necessary to closely follow the epidemiology of MRSA to be able to properly combat it efficiently. Although there are some promising new antibiotic treatments in the works, it is still critical to manage many resistance patterns by utilizing the combined strategy of infection control and antibiotic management.
3.1 Classification of Organisms

Life on Earth is very diverse, and in order to easily distinguish living organisms from one another a distinct classification system is utilized. All living organisms can be classified as being prokaryotes or eukaryotes.\(^1\)

Organisms that have organelles and true nuclei present are placed in the Eukarya domain. The domain Eukarya divides into four kingdoms that include Animalia, Plantae, Protista, and Fungi.\(^1\) Eukaryotic cells are typically the most complex in terms of their internal and external structures, and reproductive and physiological processes. One distinguishing factor of eukaryotes from prokaryotes is that eukaryotes carry out cell division through two processes of mitosis and cytokinesis.\(^1\) Additionally, eukaryotic mode of reproduction is by either mitosis or through meiosis. Mitosis is performed asexually, and meiosis is carried out through sexual reproduction.\(^1\)

The prokaryotes are a group of organisms whose cells lack membrane-bound organelles and a membrane-bound nucleus.\(^1\) Within the prokaryotes, organisms can be divided into two domains of either Archaea or Bacteria.

The cell wall of Archaea consists of pseudopeptidoglycan while Bacteria cell wall contains peptidoglycan and lipopolysaccharide.\(^1\) Further distinction of Archaea and Bacteria can be based upon the habitat of which they are found. Archaea are found in extremely harsh environments such as salt lakes, hot springs, and oceans whereas
Bacteria are found ubiquitously throughout soil, organic matter, and the bodies of plants and animals.  

3.2 Bacterium

3.2.1 Bacteria

Bacteria are a type of microscopic single-celled organism that are classified as prokaryotes. The relationship between bacteria and humans remains complex. Some bacteria prove to be beneficial, such as by curdling milk into yogurt or by helping with human digestion. Other bacteria remain very destructive and can cause serious diseases, such as those caused by pneumonia and MRSA.

Bacterial DNA floats free in a thread-like twisted structure called the nucleoid. Bacteria cells also contain circular pieces of DNA, called plasmids, that are independent of the nucleoid. Most bacterial cells have two protective coverings, an outer and inner cell membrane. However, mycoplasmas are a bacterium that do not have a cell wall at all. Bacterial cells can also have external extensions, called flagella or pili, that help with movement and adhesion (Figure 1).
3.2.2 Gram Stain

Bacteria can be classified based off of a number of different reasons, however, there are a couple of criteria that serve more beneficial in distinguishing unique types of bacteria. Bacteria can most commonly be distinguished by their shape, cell wall composition, or by differences in their genetic makeup.

Bacteria can be classified based on their cell wall composition by the Gram stain test. The Gram stain is a differential stain used to characterize bacterial organisms as
either Gram-positive bacteria or Gram-negative bacteria. The Gram stain was named after Hans Christian Gram who developed the technique back in 1884.

The first step in the gram staining procedure involves staining the bacterial cells with crystal violet, which is a purple dye. Crystal violet specifically binds to peptidoglycan which is found as a complex structure of amino acids and sugars in the cell wall of bacterial cells. Gram-positive bacteria have a thicker peptidoglycan cell wall that will allow for a stronger affinity for the crystal violet dye, than Gram-negative bacteria.

Iodine is the second step in the Gram staining procedure. Iodine acts as a mordant and forms a complex with the crystal violet dye that was applied in the previous step. Once the iodine forms the complex, the crystal violet stain is able to attach more tightly to the cell wall of Gram-positive bacteria. This is the mechanism that causes Gram-positive bacteria to stain violet. The thick peptidoglycan layer that is present in the cell walls of Gram-positive bacteria is able to retain the crystal violet-iodine complex better than Gram-negative bacteria, which is why Gram-positive bacteria stain purple.

After iodine is applied, a de-colorizer such as ethanol or acetone is used. The de-colorizer will wash the crystal violet stain from Gram-negative organisms. A decolorizer achieves its purpose by dehydrating, tightening, and shrinking the peptidoglycan layer. In doing so, large crystal violet molecules cannot penetrate the now tightened layer of peptidoglycan, and it becomes trapped in the cell wall of Gram-positive bacteria. In contrast, the outer membrane of Gram-negative bacteria cannot retain this crystal violet-iodine complex, and the color becomes lost after the application of the decolorizer.
Gram-negative bacteria stain red under the Gram stain technique (Figure 2). Since gram negative bacteria have a thinner peptidoglycan layer within their cell walls, they are unable to retain the crystal violet and iodine complex. After decolorization, a red pigmented safranin stain is applied. Safranin will turn any Gram-negative organisms red so that they can be visibly seen under a microscope. Safranin is a much lighter stain as compared to the crystal violet dye and therefore does not disrupt the purple coloration of the Gram-positive bacteria.

![Image of Gram stain reaction results](Microbeonline, 2013)

**3.2.3 Bacterial shapes**

Bacteria is typically classified into three main different kinds of shapes. Bacteria that take up a round shape are referred to as cocci (Figure 3). Cylindrical, capsule to pill shaped bacteria are called bacilli (Figure 4) and spiral shaped bacteria are termed spirilla (Figure 5).
Figure 3: Microscopic image of cocci bacteria in cluster formation. (Knorre, 2014)

Figure 4: Microscopic image of bacilli bacteria. (Knorre, 2014)
Cocci can interact and associate with one another to create different configurations. Combinations of two cocci are termed diplococcus.\footnote{A linear chain of cocci is representative of streptococci and a cluster of cocci represents staphylococci organisms. Many names of bacteria species is representative of the shapes and configurations that the bacteria take. For example, the infection-causing organism \textit{Staphylococcus aureus} is a cluster of cocci.\footnote{Most bacteria multiply and reproduce by a process called binary fission.\footnote{Binary fission is a rather simple process that involves having a cell grow to twice its starting size and then splitting into two new cells.\footnote{However, a bacterium must divide at the correct}}}}
time, in the right place, and must give each offspring a complete copy of its genetic material to remain competitive and viable for future generations.  

Therefore, understanding the mechanisms that bacteria cells undergo to complete propagation is of growing interest among investigative research laboratories. Understanding the mechanics of binary fission can allow for the design of novel antibiotics and of new chemicals that can specifically target and interfere with cell division.

Prior to beginning the binary fission process, a bacterial cell must first copy its DNA genetic material and segregate these two pieces to opposite ends of the cell. Then, many proteins including FtsZ assemble at the future division site in a ring-like structure at the center of the cell. This machinery positions in such a way as not to damage the DNA in the process of cleaving the cytoplasm. In many bacterial cells, as the division process carries out and the cytoplasm is split in two, a new cell wall is synthesized. The order and timing of these processes in DNA replication, DNA segregation, and synthesis of new cell wall are all very tightly controlled to execute a successful process.

The DNA that is found within the parent and newly formed daughter cells after binary fission is exactly the same as the parent’s. Therefore, bacterial cells attempt to introduce variation in their genetic material by incorporating additional outside DNA into their own genome. This process is known as horizontal gene transfer. The resulting genetic variation that occurs as a result of horizontal gene transfer ensures that bacteria will be able to adapt and survive as their environment is continually...
There are three ways that horizontal gene transfer can occur; by way of transformation, transduction, and conjugation.

During the process of transformation, bacterial cells are able to incorporate short fragments of external DNA from their surroundings into their own genome. These short fragments of DNA are typically released by nearby bacteria that have ruptured.

Horizontal gene transfer by the mechanism of transduction involves a bacteria cell becoming infected by a specific virus known as a bacteriophage. Bacteriophages can carry bacterial DNA and integrate itself into a host genome.

Conjugation involves physical contact between two bacterial organisms. Genetic materials, typically in the form of a duplicated plasmid, will transfer from a donor to a recipient by physical touch (Figure 6). Donor bacteria contain a sequence of DNA referred to as the F-factor that allows pilus formation between the two bacteria. Conjugation can greatly aid in the spread of antibiotic resistance.

Figure 6: Bacteria microscopically undergoing conjugation. (Lucbourne, 2016)
3.2.5 Antibiotic Resistance

Antibiotics are commonly used to treat bacterial infections.\textsuperscript{35} However, recently antibiotics have been increasingly used improperly or unnecessarily and have caused the spread of several strains of antibiotic-resistant bacteria.\textsuperscript{29}

Antibiotic resistance occurs when bacterial cells develop the ability to resist and defeat the antibiotic drugs that were designed to kill them.\textsuperscript{34} Organisms that become antibiotic resistant are then able to grow and potentially reproduce. Some cases of antibiotic-resistant organisms have now become impossible to treat.\textsuperscript{35}

In many cases, antibiotic-resistant bacterial infections can lead to additional follow-up doctor visits, an extended hospital stay, and costly, and many times toxic, alternatives.\textsuperscript{36} Antibiotic-resistance is specifically referring to bacteria that have become resistant to the antibiotics that were designed to kill them, not that a human receiving treatment is becoming resistant to antibiotics.\textsuperscript{33}

Every year, the United States has an estimated two million people become infected with antibiotic-resistant bacteria, and at least 23,000 persons die as a result.\textsuperscript{37} Bacteria will continue to strive to look for ways to resist and survive new drugs. However, today more and more bacteria are sharing their resistance with one another, which is making it harder for researchers to keep up.\textsuperscript{38}

One of the most notorious and deadly antibiotic resistant bacterial strains is methicillin-resistant \textit{Staphylococcus aureus}.\textsuperscript{38} Methicillin resistance results from the production of an alternative to the penicillin-binding protein, PBP2a. PBP2a is encoded
by the \textit{mecA} gene on the staphylococcal cassette chromosome \textit{mec}, specifically \textit{SCCmec}.

The \textit{mecA} gene encodes the low-affinity penicillin-binding protein PBP2a.\textsuperscript{38}

3.3 Staphylococcus

3.3.1 Staphylococcal Structure

Staphylococci are among the most common bacterial organisms causing joint infections, and emerging methicillin-resistant \textit{Staphylococcus aureus} strains continue to remain a global problem.\textsuperscript{39} In 1880, sir Alexander Ogston, a Scottish surgeon, was the first to show that a certain pus-forming disease was associated with a cluster-forming organism.\textsuperscript{40} He then introduced the name staphyococcus’ to his findings, now a genus name for a group of organisms that are facultatively anaerobic, catalase-positive, Gram-positive cocci.\textsuperscript{37}

Staphylococci are Gram-positive cocci with an average diameter of 0.9\textmu m (Figure 7).\textsuperscript{41} This bacterium tends to be arranged most commonly in a group of irregular clusters or ‘bunches of grapes.’\textsuperscript{42} Colonies of staphylococci are typically white with regular edges. Staphylococci are non-spore forming and non-motile, and most species are facultative anaerobes that display a fermentative metabolism.\textsuperscript{42} They are resistant to lysozyme, bacitracin, and to O/129, and are usually oxidase-negative and catalase-positive.\textsuperscript{41}
Growth of staphylococci occurs on blood and nutrient agars, but not on MacConkey agar. Most strains of staphylococci do not have a capsule, but a limited number do. There are approximately 30 strands of staphylococci found among animals, but most are not pathogenic; staphylococci organisms are considered opportunistic pathogens.

Most infections that involve staphylococci are pyogenic and acute. The two major pathogenic species of staphylococci are *Staphylococcus intermedius* and *Staphylococcus aureus*. Recent studies have identified biofilms in wounds of infected persons and have begun to highlight how biofilms impede inflammatory responses and the efficacy of antimicrobial therapy.

3.3.2 Staphylococcal Classification
Both pathogenic strains of staphylococcus, *S. aureus*, and *S. intermedius*, are coagulase-positive, which typically correlates well with pathogenicity.\(^{44}\)

Coagulase-negative forms of staphylococci mostly occur as commensal organisms within the environment.\(^{15}\) Staphylococci are often hemolytic on blood agar and salt tolerant. Identification of staphylococci organisms requires biotype analysis for confirmation.\(^{44}\)

### 3.3.3 Staphylococci Natural Habitat

Staphylococcal organisms are considered a major component of the normal microflora of humans and animals and only occasionally cause opportunistic infections.\(^{43}\) Staphylococci are widely common and present on animals; however, staphylococci can also be found and survive for long periods of time in the environment as well. Staphylococci are resistant to high salt concentrations and to dry conditions, making these organisms well-suited for the skin, which is considered their ecological niche.\(^{45}\) This organism can be found as part of the normal flora of the upper respiratory tract.\(^{41}\)

Staphylococci are found commonly on the skin of healthy individuals. *Staphylococcus aureus* is even present in the nose of up to 30% of healthy people.\(^{42}\) However, *S. aureus* can cause infections where there is a lower host resistance, such as with damaged skin or an open wound.

### 3.3.4 Staphylococcal Pathogenesis
*Staphylococcus aureus* contains many potential virulence factors including factors that inhibit phagocytosis, such as the capsule and immunoglobulin binding protein A (Figure 8). Other virulence factors include surface proteins that promote the colonization of host tissues and toxins that can damage the host tissue, and of which cause the disease symptoms.

### Virulence Factors of Staphylococcus

![Virulence Factors of Staphylococcus](image)

Figure 8: Common virulence factors of *Staphylococcus* including a capsule and protein A. (Microbiologyinfo, 2009)

Coagulase-negative staphylococci, however, are typically much less virulent and express fewer virulence factors. An exception, *S. epidermidis*, is a strain of *Staphylococcus* that readily colonizes implanted devices.

#### 3.3.5 Staphylococci Host Defense
The host defense’s major mode of action against staphylococci is by phagocytosis. The host produces antibodies that can neutralize toxins and promote opsonization of the organism. However, the capsule and protein A that some staphylococci possess can interfere with phagocytosis. Biofilm growth that occurs on implants is impervious to phagocytosis.

3.3.6 Staphylococci Treatment

Infections of staphylococci that occur outside of the hospital setting can typically be treated with penicillinase-resistant β-lactams. However; healthcare associated infections (HAIs) are often caused by antibiotic-resistant strains of staphylococci and can therefore could only be treated with vancomycin.

3.4 Staphylococcus aureus

3.4.1 Epidemiology of Staphylococcus aureus Infections

*Staphylococcus aureus* is a major causative agent of community-acquired infections. Due to this; it has become critically important to determine the relatedness of isolates collected during the investigation of a *Staphylococcus aureus* outbreak. Typing systems are needed and must be easy to interpret and use, reproducible, and discriminatory. The traditional method for typing *Staphylococcus aureus* is by phage-typing.
Phage-typing is a method based on a phenotypic marker that has poor reproducibility. Additionally, phage-typing is not able to type many isolates (20% in a recent survey conducted by the Center for Disease Control and Prevention). Phage-typing also requires a large amount of phage stocks and propagating strains which can only be performed by a select few reference laboratories. This ultimately makes phage-typing a very high maintenance typing system.

Several other molecular typing methods have now been employed to use for the epidemiological analysis of *Staphylococcus aureus*, in particular, of methicillin-resistant strains (MRSA). Another analysis tool, plasmid analysis, has begun to be used with widespread success. However, with plasmid analysis, the plasmids can become easily lost and are therefore inherently unreliable.

Other methods are designed to recognize specific restriction fragment length polymorphisms (RFLP) using an assortment of different gene probes. One of these gene probes being rRNA genes (ribotyping), which has a limited success rate in the epidemiology of MRSA. In ribotyping, researchers choose a specific restriction enzyme to cleave genomic DNA and probes at a particular mark.

Random primer PCR also offers a potential for differentiating different strains of *Staphylococcus*. However, a usable primer has not yet been identified for *Staphylococcus aureus*. The method that is currently regarded as the gold standard is pulsed field gel electrophoresis (PFGE). This method involves genomic DNA being cut by a restriction enzyme that can generate large fragments of DNA of 50-700kb (Figure 9).
3.4.2 Clinical Manifestations of Staphylococcus aureus

*Staphylococcus aureus* is a common causative agent of furuncles, sties, impetigo, boils, and other superficial skin infection in humans (Figure 10). Staphylococcal infections can also cause more serious infections, especially in patients who are debilitated by traumatic injury, chronic illness, immunosuppression, or burns.
Severe infections can include deep abscesses, endocarditis, pneumonia, phlebitis, and meningitis, and are commonly more associated with hospitalized patients rather than healthy persons within the community. S. epidermidis and S. aureus are frequent causes of infections associated with indwelling catheters, such as cardiovascular devices, joint prostheses, and artificial heart valves (Figure 11).
3.4.3 Pathogenesis of Staphylococcus aureus Infections

Staphylococcus aureus will express many virulence factors, including in the form of cell surface-associated and extracellular proteins. The role of any given virulence factor has been difficult to determine because Staphylococcus aureus pathogenesis is typically multifactorial.

The application of molecular biology has now led to significant recent advances in the awareness of the pathogenesis of staphylococcal diseases. Some advancements, such as looking at the genes that encode potential virulence factors, have been sequenced and cloned. In doing so, studies are being conducted that look at the molecular level of the potential virulence factors mode of action.
Genes that encode some putative virulence factors have also now been able to be inactivated, and the virulence of the mutant strains can then be compared to those of the wild-type strain. Any reduction in size in virulence is indicative of that missing factor. However, if virulence is restored when the gene is returned to the mutant organism, then “Molecular Koch’s Postulates” have been fulfilled. This method has confirmed several virulence factors of S. aureus.

3.4.4 Staphylococcus aureus Avoidance of Host Defenses

Staphylococcus aureus expels several different factors that have the potential to interfere with various host defense mechanisms. However, studies have shown strong evidence that these factors lack a role in the virulence of an organism.

A large majority of Staphylococcus aureus clinical isolates express a surface polysaccharide. These polysaccharide capsules are of either serotype 5 or 8. These capsules are termed microcapsule because they can only be seen under an electron microscope after antibody labeling. Additionally, S. aureus expresses high levels of polysaccharide that is rapidly lost upon laboratory subculture. The function of the capsule remains unclear.

Staphylococcus aureus also contains a surface protein, protein A. Protein A is able to bind to immunoglobulin G molecules by the Fc region (Figure 12).
Figure 12: The host defense mechanism of S. aureus protein A binding to immunoglobin G via the Fc region. (Angert, 2005)

In principle, the mechanism of this molecule will disrupt phagocytosis and opsonization. Mutants of S. aureus that lack protein A are more effectively phagocytized. Similarly, other studies have shown that mutant organisms that have protein A present have enhanced virulence.

3.4.5 Staphylococcus aureus Damage to the Host

Staphylococcus aureus can express many different kinds of protein toxins that are likely responsible for the symptoms that occur during an infection. Some of these proteins cause damage to the membranes of erythrocytes, causing hemolysis. The leucocidin that many S. aureus express is likely the cause of membrane damage to leukocytes. This leukocyte membrane damage is not hemolytic. Systemic release of the
α-toxin is the cause of septic shock. Enterotoxins and TSST-1 can cause toxic shock of an infected individual.\textsuperscript{53}

\textbf{3.4.6 Staphylococcus aureus Superantigens}

\textit{Staphylococcus aureus} is able to express two different types of toxins with superantigen activity. One of which being an enterotoxin that has six serotypes (A, B, C, D, E, and G) and the second being toxic shock syndrome (TSST-1).\textsuperscript{56}

Enterotoxins are responsible for staphylococcal food poisoning and can cause vomiting and diarrhea when ingested. Enterotoxins can also cause toxic shock syndrome (TSS) when expressed systematically. Enterotoxins B and C cause 50% of non-menstrual TSS.\textsuperscript{56}

TSST-1 does not have any emetic activity. Toxic shock syndrome can occur from any staphylococcal infection if TSST-1 or an enterotoxin is released systemically, and the host lacks needed neutralizing antibodies.\textsuperscript{52}

T cells are stimulated by super antigens and non-specifically without normal antigenic recognition (Figure 13).\textsuperscript{13}
A superantigen can activate up to one in five T cells, whereas only one in 10,000 are actually stimulated during antigen presentation. Cytokines are released which cause symptoms similar to TSS. Super antigens are able to directly bind to class II major histocompatibility complexes of antigen-presenting cells. This complex is, in turn, able to recognize only the Vβ element on T cell Receptors, allowing any T cell with the appropriate Vβ element to be stimulated (Figure 14). Typically, antigen specificity is also needed for successful binding.
3.4.7 Resistance of Staphylococci to Antimicrobial Drugs

*Staphylococcus aureus* strains that are associated with hospital settings are often resistant to several different antibiotics. There have even been several strains discovered that are resistant to all clinically useful drugs. The term MRSA specifically refers to methicillin resistant strains of *Staphylococcus aureus*. Additionally, *S. aureus* also expresses resistance to many disinfectants and antibiotics, such as quaternary ammonium compounds. This may be one mode that allows *Staphylococcus aureus* strains to survive better within the hospital environment.
Since the start of when antibiotic use began to increase, *S. aureus* has continued to rapidly acquire resistance by a variety of genetic mechanisms. One of these mechanisms involves the acquisition of additional genetic information or extrachromosomal plasmids in the chromosome via transposons or other types of DNA insertion. Another method by which *S. aureus* acquires antibiotic resistance is by mutations in chromosomal genes.

There are fundamentally four mechanisms of which resistance to antibiotics in bacteria occurs (Figure 15). The first involves enzymatic inactivation of the drug. Alterations to the drug target to prevent binding is another method, as well as accelerated drug efflux. This mechanism prevents toxic concentrations from accumulating in the cell. The last method involves a by-pass mechanism whereby an alternative drug-resistant version of the target is able to be expressed.

Figure 15: The four mechanisms of which resistance to antibiotics in bacteria can occur. (ResearchGate, 2015)
3.4.8 Community-Associated MRSA

Community-associated MRSA infections (CA-MRSA) are a type of MRSA infection that can occur in healthy people who have not had a medical procedure or have not been hospitalized within the past year.\textsuperscript{58} Recent outbreaks of CA-MRSA have been most prevalent among prisoners, athletes, daycare attendees, military recruits, and groups of people who routinely share contaminated items or live in a crowded setting.\textsuperscript{14} Lack of hand washing can also lead to the spread of bacteria more easily and can make persons more susceptible to CA-MRSA.\textsuperscript{21}

CA-MRSA infections typically begin as skin infections and can usually be treated by providing localized care. However, if left untreated, CA-MRSA infections can eventually progress into a much more serious complication.\textsuperscript{14} CA-MRSA is spread in the same way that MRSA is spread, and it is important to cover skin infections that have been caused by MRSA until they are fully healed. People who exhibit close contact, especially family members, should wash their hands with soap and water frequently.\textsuperscript{59}
CHAPTER 4

Procedure

4.1 Specimen Collection

To collect the numerous specimens needed for this experiment, BBL™ CultureSwab™ EZ collection and transport system specific swabs were distributed to participants. The BBL™ CultureSwab™ EZ systems are self-contained, ready-to-use specimen collection systems that provide a simplified method for the transport and maintenance of viable microorganisms (Figure 16).60

![Figure 16: BBL™ CultureSwab™ EZ collection and transport system.](Copan, 2017)

The culture swab devices incorporate a polyurethane-tipped swab on a plastic shaft that is secured to a cap. The swab contained within the cap is inserted into a tube. Studies have shown that BBL™ CultureSwab™ EZ systems yield equivalent results as compared to traditional fiber-tipped collection swabs placed in transport media.60

For isolation and identification of aerobic microorganisms, the specimen must be removed from the culture swab transport container and cultured on appropriate media or
processed as appropriate for other microbiological procedures. The culture swabs contain Amies liquid media that allows the transport system to maintain viability of aerobic, anaerobic, and fastidious bacteria at room and refrigerator temperature for up to 48 hours.

Each household was provided with four sterile culture swabs that were placed into one individual baggie with guided instructions on the collection process (Figure 17). Specific labels were also contained in the baggie that included a unique sample set number and a letter in reference to the surface that the sample was to be collected from. The four high-touch surfaces where each given a corresponding letter to ensure anonymity: (A) Inside front door handle, (B) Refrigerator door handle, (C) Remote control, and (D) Kitchen sink handle. Participants were told to swab an approximate 2 x 2-inch surface area. Once the participants were given a baggie and instructions, they were given two days, or up until 48 hours of receipt of the collection preparation baggie, to return their collection sample swabs. Swabs that were not returned within this time frame were discarded and not included in this study.
On a separate spreadsheet, the entire collected sample set numbers, and corresponding names and phone numbers if the participants included this information, were kept in case MRSA was isolated from a participant’s home. If MRSA was isolated from a participant’s sample swab, then that participant was contacted if they provided contact information and instructed on how to disinfect the high-touch surface. A total of 304 swab samples were collected from 76 different homes within the central Texas region. Each home environment had four different areas within the home that were swabbed. The areas included the inside front door handle, refrigerator door or pull handle, the most used remote control, and the kitchen sink handle.
4.2 Specimen Plating

Traditional methods for the identification of MRSA were carried out for this experiment. Once the baggies that contained the four swabs, four labels, and instructions were given to participants, each swab specimen that was returned was first plated onto a BD BBL™ prepared mannitol salt agar (MSA) media plate (Figure 18). The MSA plates were inoculated by rolling the swab over the plate to ensure a thorough inoculation of the swabbed specimen. The swabs were plated onto the MSA agar plate within 48 hours of distributing the collection packs to the participants, and within 4 hours of receiving the collection pack from the participants. This ensured that no collected swabbed went un-plated past 48 hours.

![BD BBL™ prepared mannitol salt agar (MSA) media plate with organism growth on it.](Fischersci., 2012)

Each MSA plate was split into four evenly distributed quadrants (Figure 19). Each quadrant was labeled with the letter that corresponded with the area that the swab was
collected from (i.e. Quadrant ‘A’ is where the swab that collected specimen from the inside of the front door handle was plated). This allowed each home to be plated on one MSA plate (i.e. The four quadrants of the MSA plate equated to the four areas of the house that the specimens were collected from).

Figure 19: An MSA plate exhibiting the four quadrants split to fit one household’s swabs onto a single plate.

Mannitol salt agar is used for selective isolation and enumeration of staphylococci from clinical and nonclinical materials.\textsuperscript{62} MSA is a selective and differential medium. Mannitol salt agar contains a high concentration of salt, 7.5% sodium chloride
concentration, that allows for the media to have selective growth for organisms of the genus *Staphylococcus*.\(^6^3\) *Staphylococcus* organisms are able to tolerate high saline levels, which allows them to grow well on MSA. Organisms that come from other genera may also grow on MSA, but they typically grow very weakly.\(^6^3\)

Mannitol salt agar also contains the pH indicator phenol red and the sugar mannitol.\(^6^4\) If an organism is able to ferment mannitol, an acidic byproduct will form that will cause the phenol red incorporated in to the agar to turn a yellow pigment.\(^6^5\) Most pathogenic staphylococci, such as *Staphylococcus aureus*, are able to ferment mannitol. In contrast, most non-pathogenic staphylococci are not able to ferment mannitol.\(^6^5\)

Once the MSA plates were inoculated, the MSA plates were incubated at 37°C for 16 to 24 hours. A sterility confirmation plate was included throughout the incubation process for quality control purposes. After the 24-hour incubation period, the sterility confirmation plate remained negative, or no growth. Colonies that turned yellow on the MSA plate after the 24-hour incubation time were presumptively identified as *Staphylococcus aureus*.

### 4.3 Species Identification

Once the collected specimens were plated on MSA and incubated for 24 hours, the suspected *Staphylococcus aureus* colonies, which were representative of a yellow colony, a catalase test was performed. The catalase test was performed by using a glass slide and 3% hydrogen peroxide (Figure 20). All yellow colonies that were inoculated
onto the slide and 3% hydrogen peroxide that produced bubbles, indicating a positive catalase test, were then tested for coagulase.\textsuperscript{68}

![Figure 20: Positive and negative result for the catalase test on a glass side using 3% hydrogen peroxide.](Studyblue, 2004)

The Remel\textsuperscript{TM} Bactistaph\textsuperscript{TM} Latex Agglutination Kit was used to test the yellow colonies that tested positive for catalase, for coagulase activity (Figure 21). This test is a latex slide agglutination test used to differentiate \textit{S. aureus} from other \textit{Staphylococcus} species by the detection of the protein A clumping factor.\textsuperscript{66}

![Figure 21: Remel\textsuperscript{TM} Bactistaph\textsuperscript{TM} Latex Agglutination Kit](Fischersci., 2008)
The cell wall polypeptide clumping factor, protein A, is in most strains of \textit{S. aureus}.\textsuperscript{66} Protein A is found on the cell surface of approximately 85\% of human strains of \textit{S. aureus}, and this protein has the ability to bind the Fc portion of immunoglobulin G (IgG).\textsuperscript{67} For this test, agglutination will occur if the organism is \textit{S. aureus}. On the contrary, if another species is present other than \textit{Staphylococcus}, then the result is a possible string-like or thread-like pattern, that should be carefully distinguished from a clumping result.\textsuperscript{66}

The principle of the Remel™ Bactistaph™ Latex Agglutination Test is that the kit consists of blue latex particles that are coated with human fibrinogen and IgG.\textsuperscript{67} Upon mixing the latex with colonies of staphylococci, which have protein A or clumping factor present, cross-linking will occur creating visible agglutination of the latex particles (Figure 22). If neither Protein A or clumping factor are present, then no agglutination will occur, and the result is to be determined as negative.\textsuperscript{67}

![Image of Remel™ Bactistaph™ Latex Agglutination Test results]

\textbf{Figure 22:} Positive clumping results and negative result for the Remel™ Bactistaph™ Latex Agglutination Kit for the coagulase test.

(Image from M. Tupper & K. Vickers “MRSA in the Home” senior project presentation, 2018)
4.4 MRSA Confirmation

Strains of *S. aureus* that are oxacillin and methicillin resistant, are considered and termed methicillin-resistant *S. aureus*. The accepted screening method for MRSA, as determined by the Clinical and Laboratory Standards Institute (CLSI), recommends a plate containing 6 µg/ml of oxacillin in Mueller-Hinton (MH) agar supplemented with NaCl (4% w/v; 0.68 mol/L) as the method of testing for MRSA.\textsuperscript{67} We performed an accepted adopted method that utilizes Mueller-Hinton agar with a 1 µg oxacillin disk placed on a lawn of inoculated specimen.

All isolates that tested positive for both catalase and coagulase were plated onto Mueller-Hinton agar in a lawn that was overlaid with an oxacillin disk placed in the center of the lawn. The plates were incubated at 37°C for 24 hours. After the 24 hour incubation period, the zones of inhibition were measured in millimeters. A zone of inhibition that was greater than 13mm was considered susceptible. A zone of inhibition 11-12mm was considered intermediate and a zone of inhibition that was less than or equal to 10mm was considered resistant (Figure 23).
Confirmed *Staphylococcus* species that were not susceptible to oxacillin on Mueller-Hinton agar were then subcultured onto HardyCHROM™ MRSA chromogenic agar from the individual colonies on MSA plates (Figure 24). HardyCHROM™ MRSA agar is a selective and differential media that allows for the growth of MRSA colonies.70
HardyCHROM™ MRSA is a selective and differential culture medium that facilitates the isolation and identification of methicillin-resistant *Staphylococcus aureus* (MRSA) to help aid in the prevention and control of MRSA infections. This chromogenic medium simplifies the identification of MRSA infections. MRSA strains grown in the presence of the chromogenic substrates incorporated into the agar produce deep pink to magenta colonies, called mauve colonies. Color development is bright and distinct to allow for easy reading.

The principle behind the chromogenic agar plate is that when the target organism’s enzyme cleaves the colorless chromogenic conjugate, the chromophore is
released. When the chromophore is its unconjugated form, the chromophore exhibits its distinctive color and, due to reduced solubility, forms a precipitate.

The HardyCHROM™ MRSA contains cefoxitin as well as other inhibitory agents that will suppress the growth of other nonresistant organisms. This will allow for the proliferation of MRSA. MRSA colonies will turn mauve to purple in color after a 24 incubation time, which indicates a positive MRSA isolate.

Throughout the inoculation and incubation of isolates on the CHROMagar plates, exposure to light was kept at a minimum as the plates are light sensitive. To help ensure that light exposure was minimalized, the plates were warmed to room temperature in the manufacturer’s packaging and then placed directly into the 37°C incubator upon inoculation. A positive CHROM agar reaction can range from light pink to mauve colonies and is seen only in methicillin resistant strains of *Staphylococcus*.

### 4.5 Verification Testing

To be thorough, the two isolates that did not show resistance to the oxacillin disk on Mueller-Hinton agar that showed no growth on the CHROMagar were sent to the Central Texas Medical Center (CTMC) for confirmation. These two isolates were collected by M. Tupper and K. Vickers and the images of the organisms examined during microscopy were taken and sent back for our records. If the images were non-Gram positive cocci they were not considered MRSA and no additional testing was performed.

Further additional confirmation testing that can be performed can include the utilization of the VITEK®2 antibiotic susceptibility testing system. If time allows,
isolates that were confirmed as MRSA by displaying a mauve to purple color on the HardyCHROM™ MRSA agar plate after 24 hours of incubation will be tested on the VITEK®2 antibiotic susceptibility testing system (Figure 25). VITEK®2 testing can be performed on all suspected MRSA isolates at a local hospital laboratory.

![VITEK®2 antibiotic susceptibility testing system](image)

**Figure 25:** VITEK®2 antibiotic susceptibility testing system

(Biomerieux, 2001)

The VITEK®2 antibiotic susceptibility testing system is used for the microbial identification of bacteria and yeast. The VITEK®2 is also used for antibiotic susceptibility (AST) and resistance mechanism detection. A sterile swab or applicator stick is used to transfer a sufficient amount of colonies of a pure culture. Once transferred, the microorganism is suspended in 3.0 mL of sterile saline and measured using a turbidity meter. Identification cards are then inoculated with microorganism suspensions using an integrated vacuum apparatus. A complete list of all the organisms that the VITEK®2 can test for on the Gram positive identification card are presented in Appendix A.
A transmittance optical system allows for the interpretation of test reactions using different wavelengths in the visible spectrum. During the incubation time, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism.\textsuperscript{71}
CHAPTER 5

Results and Discussion

5.1 Staphylococcus Characterization

One hundred and four samples were collected during the Spring of 2018 and 200 samples were collected during the Spring of 2019. The total sample size consisted of 76 homes across the central Texas area for a total of 304 specimens collected from four, high-touch surfaces of each home. Out of the 304 culture swab specimens collected that were first plated onto mannitol salt agar (MSA), 118 showed growth (118/304, 38.8%). Of the 118 samples that showed growth, 92 (92/304, 30.3%) tested positive for S. aureus and were presumptively identified as S. aureus. Table 1 shows the percentage of presumptive Staphylococcus aureus that was isolated from each surface source (Table 1).

Table 1: Shows the percentage of presumptive Staphylococcus aureus from each of the four high-touch surface sources.

<table>
<thead>
<tr>
<th>Source</th>
<th>Samples positive for Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside Front Door Knob</td>
<td>(20/76)= 26.3%</td>
</tr>
<tr>
<td>Refrigerator Door Handle</td>
<td>(17/76)= 22.4%</td>
</tr>
<tr>
<td>Remote Control</td>
<td>(23/76)= 30.3%</td>
</tr>
<tr>
<td>Kitchen Sink Knob</td>
<td>(32/76)= 42.1%</td>
</tr>
<tr>
<td>Total: All Sources</td>
<td>(92/304)= 30.3%</td>
</tr>
</tbody>
</table>

The kitchen sink knob had the most positive samples presumptively identified as inhabiting S. aureus, with 42.1% (32/76) of all of the kitchen sink knob samples being positive. Out of all 304 samples that were collected from the 76 different homes, 92 samples were positive for presumptive S. aureus. However, the overall presence of S.
*aureus* alone is not alarming because *Staphylococcus* species can be commonly found on the skin as normal flora. The samples that were positive for presumptive *S. aureus* do not yet indicate that the organism displays antibiotic resistance.

### 5.2 MRSA Prevalence

Out of the 92 samples that were presumptively positive for *S. aureus*, only nine isolates had zones of inhibition less than 13mm when plated on Mueller-Hinton agar with an oxacillin disk. Out of those nine isolates, five had zones of 12mm, two had a zone of 10mm, and 2 had no zone of inhibition present (Table 2; Figure 26).

<table>
<thead>
<tr>
<th>Oxacillin Results on Mueller Hinton agar</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant ≤ 10mm</td>
<td>(4/92)= 4.3%</td>
</tr>
<tr>
<td>Intermediate 11-12mm</td>
<td>(5/92)= 5.4%</td>
</tr>
<tr>
<td>Susceptible ≥ 13mm</td>
<td>(83/92)= 90.2%</td>
</tr>
</tbody>
</table>

Figure 26: Pie chart of the oxacillin disk results on Mueller-Hinton agar.
The nine isolates that had zones of inhibition less than 13mm were plated onto CHROMagar. Of those nine isolates, three showed mauve to pink colored colonies and were confirmed as MRSA. Four of the plated organisms grew blue colonies and were not considered MRSA. The remaining two isolates did not show any growth after 24 hours of incubation. All three of the confirmed MRSA isolates were from swabs collected from the remote control high-touch surface.

To be thorough throughout the identification process, the two samples that did show oxacillin resistance on Mueller-Hinton agar but did not grow on CHROMagar, which were both collected during the Spring of 2018, were sent to the Central Texas Medical Center (CTMC) for confirmation. Both of the two samples showed organisms that were gram positive/gram variable rods (Figures 27-28).

Figure 27: Gram stain photo that was taken at CTMC of an isolate. (Photo was taken by Eric Williams at CTMC, 2018)
5.3 Limitations

Ideally, all of the isolates that showed oxacillin resistance would have grown on CHROMagar to be confirmed as MRSA. However, this was not the case. This could be due to the fact that the latex agglutination test that was used to determine the coagulase activity of the isolates tests for both clumping factor and protein A. Some of the organisms that can cause false positives for this test include *Staphylococcus saprophyticus, Staphylococcus lugdunensis, and Staphylococcus schleiferi*. Additionally, the latex agglutination test that was utilized for this study recommends Gram staining of the organism prior to testing. Gram staining could have eliminated further work-up of organisms that were Gram positive rods. Oxacillin is a beta-lactam antibiotic and Gram positive rods can demonstrate antibiotic resistance to beta-lactams.
5.4 Discussion of Results

Similar studies have been conducted to quantify the prevalence and characterization of *Staphylococcus* species, including MRSA, in the home. A previous study conducted by T. Mann and R. Sturgis from a past cohort of the Texas State University Clinical Laboratory Science Program conducted similar research. In this study, they collected swab samples from high-touch surfaces from 20 homes across Texas. The surfaces in their research experiment were slightly different in that in this experiment a refrigerator handle was substituted for a toilet handle from their study. The results from their study confirmed four isolates of MRSA that were all isolated on remote controls.

A possible explanation as to why the remote control demonstrated the most MRSA isolates is that the remote control is handled by more individuals and more frequently that the other high-touch surfaces. Additionally, the remote control is the only high-touch surface in this experiment that allowed for the individual to pick up the object before or during the collection of their swab specimen. This could have contributed to more viable organisms being present during the time of collection.

The results to this study are similar to those from past experimentation, showing a somewhat qualitatively longitudinal analysis of MRSA isolation in homes. Since the isolation of MRSA was confirmed, it is recommended that proper disinfection techniques are performed with effective materials. Adequate disinfection requires the use of a detergent based cleaner. Depending on the disinfectant that is used, a specific protocol for proper disinfection must be utilized. The protocol typically includes contact time,
organisms targeted, and certain precautionary measures. Many disinfectants are common and can be purchased at a local grocery store.

5.4 Summary of Results

A total of 304 swab samples from 76 different households were collected from four different high-touch surfaces of each home. 118 (118/304, 38.8%) samples showed microbial growth on MSA and 92 (92/304, 30.3%) samples tested positive presumptively for *S. aureus* by having positive catalase and coagulase reactions. Of those 92, four demonstrated oxacillin resistance by having a zone of inhibition less than or equal to 10mm and three (3/92, 3.4%) were confirmed as MRSA by growing mauve to pink colonies on CHROMagar. The findings of this study indicate potential exposure risks from *Staphylococcus* species, including MRSA, in everyday home environments and demonstrate the need for proper disinfection to avoid infection of at-risk individuals, such as immunocompromised persons.
CHAPTER 6

Conclusion

6.1 Summary of Research

This study assessed the prevalence of *Staphylococcus*, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), on four high-touch surfaces in the home environment. The findings of this research demonstrate that MRSA can be found on high-touch surfaces of the home. This research also demonstrates the growth of other *Staphylococcus* species that can often be found on four common high-touch surfaces of homes across central Texas. Many staphylococcal organisms are often present as normal flora on human skin and will not cause any serious infections, however, MRSA can pose a serious as it is resistant to commonly used antibiotics.

Emerging methicillin-resistant *Staphylococcus aureus* strains continue to remain a global problem, and their prevalence in the community, specifically the home, is demonstrated by this study. Many hard surfaces are known to serve as good reservoirs for MRSA, including door knobs. The findings of this study support the growing body of evidence that the environment (surfaces) plays an important role in the transmission of pathogens, specifically MRSA, in the community.

6.2 Summary of Conclusions and Implications

Due to MRSA being able to rapidly spread, and frequent hand and skin-to-skin contact among individuals in a home, certain groups may be at an increased risk for exposure to MRSA infections do to their cultural habits and interactions with one another.
and with their home environment. Groups of individuals that live in warmer climates are more likely to have more skin exposure due to wearing less clothing material (i.e. wearing shorts vs. pants), putting them at an increased risk of exposure. Other high-risk groups include homes that have individuals who work in healthcare and frequently interact with patients in a healthcare setting.

The findings of this study indicate potential exposure risks from *Staphylococcus* species, including MRSA, in everyday home environments. The general public can use the information from this study, focusing on the understanding of the prevalence of MRSA and other infectious agents, to be more aware of, and engage in, proper hygiene techniques to lower their exposure risk.

6.3 Recommendations for Future Research

Future studies can look at additional high-touch surfaces that are frequently seen in the home. Such surfaces could include mattresses, toothbrushes and other daily hygiene materials, and electronics such as laptops, desktops, phones, and tablets. There is a growing body of evidence to support the contribution of surfaces to disease transmission. This supports comprehensive disinfecting routines to reduce the risk of acquiring an infection from a pathogen.

This research project could also focus on a longitudinal approach that focuses on the improvement that proper disinfecting procedures can have on high-touch surfaces. It could be beneficial to re-collect specimens from the household surfaces that tested positive for *S. aureus* and MRSA six months to a year from the initial collection and testing, and then compare the results to see if colonization improvement was achieved.
Experimentation should continue to focus on common ‘superbugs’ that are rapidly becoming more antibiotic resistant, such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae* (CRE) and vancomycin-resistant *Enterococcus* species (VRE). Studies can look at their prevalence in both the hospital and community setting as well as across different geographical regions across the United States and World. Using this data, researchers can begin evaluating the severity of the damage that these organisms can cause and make the general public, as well as healthcare professionals, more aware of their prevalence in an attempt to reduce excessive antibiotic use. Research that also focuses on creating additional drugs and antibiotics that can eliminate these organisms if infected is also of growing interest and need.
DISCLOSURE STATEMENT

There is no potential conflict of interest to report.
**APPENDIX A**

Table 3: Table 3: Bacteria that can be identified with the VITEK®2 Gram positive ID card

<table>
<thead>
<tr>
<th>Abiotrophia defectiva</th>
<th>Helcococcus kunzii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerococcus urinae</td>
<td>Kocuria kristinae</td>
</tr>
<tr>
<td>Aerococcus viridans</td>
<td>Kocuria rhizophila</td>
</tr>
<tr>
<td>Alloiococcus otitis</td>
<td>Kocuria rosea</td>
</tr>
<tr>
<td>Kytoococcus sedentarius</td>
<td>Kocuria varians</td>
</tr>
<tr>
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*Organism of interest*
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