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The Presence of Methicillin Resistant Staphylococcus aureus (MRSA) on Environmental Surfaces in Healthcare Facilities Pre- and Post-Cleaning

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THE PRESENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ON
ENVIRONMENTAL SURFACES IN HEALTHCARE FACILITIES PRE-AND POST- CLEANING

by

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2007

A thesis submitted in partial fulfillment
of the requirements for the

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ABSTRACT

The presence of methicillin resistant *Staphylococcus aureus* (MRSA) on environmental surfaces in healthcare facilities pre-and post- cleaning

by

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The emergence of antibiotic resistant bacteria is a serious public health issue affecting millions of people. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of healthcare acquired infections today. Research has shown that patients infected or colonized with MRSA shed the bacteria into the environment, where the bacteria can survive for long periods of time and become potential sources of infection. Currently, infection prevention efforts focus on active surveillance, hand hygiene, personal protective equipment use and antimicrobial stewardship, with less attention given to environmental cleaning. Nevertheless, environmental cleaning is essential to remove infectious agents from environmental surfaces and prevent transmission. The purpose of this study was to examine the effectiveness of environmental cleaning programs in removing MRSA from environmental surfaces at an acute care hospital and a long-term care facility. Environmental swabs or sponge samples were collected from five locations (floor, television remote, call bell, bathroom doorknob and bed rail) pre-

and post-terminal cleaning from the rooms of patients infected or colonized with MRSA. These swabs or sponges were then analyzed through culture isolation and polymerase chain reaction (PCR) to confirm the presence of MRSA. A total of 120 environmental samples were obtained from two medical facilities. Culture analysis followed by confirmatory PCR analysis for the *mecA* gene showed that 18 of the 120 samples (15%) were positive for the presence of MRSA of which 16 were collected pre-cleaning (89%), and 2 were collected post-cleaning (11%). The location of the positive pre-cleaning samples included the floor (5), call bell (4), bed rail (4), bathroom doorknob (2) and TV remote (1), and the positive post-cleaning samples included the floor (1) and call bell (1). The results confirm the presence of MRSA on surfaces in patient rooms and suggest that environmental cleaning was effective, but there is potential for rooms to remain contaminated. Future research should focus on identifying the most effective cleaning programs to ensure rooms are safe for patients.

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CHAPTER 1

BACKGROUND

Throughout much of human history, bacterial infections have been a leading cause of death. Before the discovery of antibiotics, the mortality rate from bacteremia caused by *Staphylococcus aureus* was above 80% (Skinner & Keefer, 1941). This began to change in 1928, when Alexander Fleming discovered penicillin, the first antibiotic (Ligon, 2004). For the first time, humans had an effective way to treat bacterial infections. This discovery saved millions of lives and changed science forever. Unfortunately, the success did not last. Bacteria rapidly developed resistance to penicillin, and it became clear that researchers were going to have to develop new types of antibiotics and understand the mechanisms of antimicrobial resistance to control infections (Chambers & Deleo, 2009).

The emergence of antimicrobial resistant bacteria is a serious public health issue affecting millions of people worldwide. Today, one of the most common types of antimicrobial resistant bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA). This bacterium is commonly referred to as “staph” and is a leading source of healthcare acquired infection in the United States and many other countries (Diekema et al., 2001). Based on prevalence rates in 2005, it has been estimated that there are 1,300,000 MRSA infections per year in the United States alone (Klevens et al., 2007). This is noteworthy because, when compared to infections caused by methicillin susceptible *Staphylococcus aureus* (MSSA) strains, MRSA infections have been demonstrated to be more difficult to

treat, increase morbidity and mortality rates, and increase medical costs (Filice et al., 2010).

Since MRSA was first identified, it has undergone several fundamental changes. Initially, infections caused by MRSA were primarily observed in populations that had been exposed to healthcare facilities and were thought to be a nosocomial problem (David & Daum, 2010). This began to change during the 1990s, when infections caused by MRSA began to emerge in the community in healthy populations with no exposure to healthcare facilities or other risk factors (Berman, Eisner, & Kreiswirth, 1993; Dammann, Wiens, & Taylor, 1988; Nimmo & Coombs, 2008; Pate, Nolan, Bannerman, & Feldman, 1995). This new type of community-acquired MRSA substantially increased the number of infections and quickly spread across the globe.

Both healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) are a leading cause of healthcare-associated infections (HAIs) in acute-care hospitals (ACH) and long-term care facilities (LTCF). MRSA infections remain a chief focus of infection prevention programs. Currently, MRSA prevention efforts have focused on populations with high prevalence of MRSA infections and healthcare facilities. Infection prevention efforts at healthcare facilities have focused on improving active surveillance to identify infected or colonized patients, hand hygiene, the use of personal protective equipment, and antimicrobial stewardship, with less attention given to environmental cleaning. Nevertheless, none of these prevention tools are effective alone, and environmental cleaning is essential in controlling the spread of bacteria (Otter, Yezli, & French, 2011). Research has shown that patients infected or colonized

with MRSA shed the bacteria into the environment (Boyce, Potter-Bynoe, Chenevert, & King, 1997; French et al., 2004; Sexton, Clarke, O'Neill, Dillane, & Humphreys, 2006), where the bacteria can survive for long periods of time (Kramer, Schwebke, & Kampf, 2006). These bacteria that are shed onto environmental surfaces become potential sources of infection for susceptible patients who spend time in healthcare facilities. Few studies have been conducted to determine the effectiveness of environmental cleaning in removing MRSA from different environmental surfaces in healthcare facilities.

CHAPTER 2

INTRODUCTION

Emergence of Antimicrobial Resistant Bacteria

It is essential to understand how antimicrobials act before examining the mechanisms of antimicrobial resistance. In general, antimicrobials inhibit bacterial infection by disrupting essential actions in the bacteria, including (1) cell wall synthesis, (2) protein synthesis, (3) nucleic acid synthesis, and (4) metabolic pathways (Neu, 1992). If an antimicrobial can inhibit, stop or alter any of these essential functions, the bacterium will be disabled or will die.

The first antimicrobial resistant bacteria were observed shortly after the clinical implementation of penicillin (Forbes, 1949; Kirby, 1944). During this time, researchers knew little about the mechanisms of resistance, but over the past 50 years scientific understanding has tremendously increased. It is now known that bacteria have the ability to develop resistance to antimicrobials through two main mechanisms: (1) natural selection of spontaneous mutations in their DNA, and (2) the incorporation of genetic material into their DNA from other bacteria (Tenover, 2006).

Spontaneous mutations commonly occur in bacteria, and occasionally the new mutation will result in the development of resistance to antimicrobials. A good example of this mechanism occurs in penicillin-resistant *pneumococci*, which have evolved resistance due to a mutation that alters the binding site for penicillin, making it ineffective (Contreras-Martel, Dahout-Gonzalez, Martins Ados, Kotnik, & Dessen, 2009).

Bacteria can also develop resistance by obtaining genetic material that encodes the resistance trait from bacteria of the same species or from different species. This genetic material can be obtained through transformation, conjugation or transduction (McManus, 1997; Tenover, 2006). Transformation occurs when bacteria obtain genetic material released from cells, often through cell lysis, and is present in the environment. Conjugation occurs when plasmids, mobile genetic elements, are directly transferred between two organisms. This can occur by mechanisms such as a pilus, which can connect two cells, or when cells conjoin during sexual reproduction. Transduction occurs when genes are transferred between bacteria by a bacteriophage, which is a virus that infects bacteria and can occasionally transfer genetic material.

Both mechanisms of resistance have been enhanced by the misuse and overuse of antibiotics (Chambers & Deleo, 2009). Antimicrobial use increases the development of resistance strains of bacteria because the antimicrobial will kill all the naturally susceptible bacteria. This allows only the bacteria that have developed resistance to survive and multiply. Current infection prevention efforts are focusing on antimicrobial stewardship to limit the misuse of antimicrobials.

Overall, bacteria have been extraordinarily successful at developing resistance to antimicrobials. Several species of antimicrobial resistant bacteria have become endemic in healthcare facilities, including *staphylococci*, *enterococci*, *Klebsiella pneumonia*, and *Pseudomonas* ("National Nosocomial Infections Surveillance [NNIS] System Report, data summary from January 1992 through June 2004, issued October 2004," 2004). Additionally, many organisms have now become resistant to multiple types of drugs,

including MRSA, vancomycin-resistant enterococci and several gram-negative bacilli.

These multi-drug resistant organisms are particularly of concern because there are seriously limited treatment options. What is most alarming is that some of the extended spectrum β -lactamase producing bacteria have been shown to be resistant to all currently available antimicrobial agents (Siegel, Rhinehart, Jackson, & Chiarello, 2007).

Methicillin Resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a ubiquitous bacterium that has plagued humans for thousands of years. *S. aureus* is a gram positive bacterium that is part of the *Micrococcaceae* family and can be characterized by its spherical shape and golden color. It can grow in both aerobic and anaerobic conditions and is often identified through positive coagulase, mannitol-fermentation and deoxyribonuclease test results (Lowy, 1998; Mahon & Manuselis, 1995).

It is necessary to note that *S. aureus* is naturally susceptible to all antimicrobials, but it is also exceptionally efficient in developing resistance. The first strains of antimicrobial resistant *S. aureus* were observed shortly after the introduction of penicillin for clinical use (Kirby, 1944). These strains became resistant to penicillin by producing a plasmid-encoded penicillinase, called β -lactamase that can break down the β -lactam ring of penicillin, making it ineffective (Chambers & Deleo, 2009).

Researchers had to create new antimicrobials to combat this new penicillin resistant organism, and were able to do so when they created a new synthetic penicillin called methicillin. Methicillin helped control the penicillin resistant strains, but the success did not last. In 1961, the first methicillin-resistant *Staphylococcus aureus* (MRSA)

strains were identified (Barber, 1961; Jevons, 1961). Interestingly, this was only two years after the introduction of methicillin.

Today, it is known that MRSA is a subclass of *S. aureus* that has developed resistance to several classes of antibiotics including penicillins, cephalosporins and carbapenems (Chambers & Deleo, 2009). Research has shown that methicillin susceptible *S. aureus* (MSSA) is able to develop resistance to antimicrobials containing a β -lactam ring because of its ability to acquire the resistance encoding *mecA* gene. The *mecA* gene is acquired from a mobile genetic element from other *Staphylococcus* species, which naturally contain this resistance gene (Moellering, 2012). The *mecA* gene produces resistance because it encodes the penicillin binding protein 2a (PBP 2a), which improves cell wall synthesis in the presence of β -lactam antimicrobials (Hartman & Tomasz, 1984; Song, Wachi, Doi, Ishino, & Matsushashi, 1987; Utsui & Yokota, 1985).

Genetic analysis of MRSA has found that the *mecA* gene is carried on a mobile genetic element that researchers have named the staphylococcal cassette chromosome *mec* (*SCCmec*) (Katayama, Ito, & Hiramatsu, 2000). Currently, there are nine different types of *SCCmec* elements (types I-VIII and V_T) that have been identified (David & Daum, 2010). While all of these nine mobile cassettes contain the complete *mecA* gene, each has a slightly different genetic makeup that can change gene expression, virulence and other characteristics. Bacteria can be characterized below the species level by strain typing, which identifies differences in the DNA between bacteria of the same species.

One important variable genetic element that several of the strains of MRSA carry is the Panton-Valentine leukocidin (*PVL*) gene. This gene is found in many community-

associated MRSA strains and is thought to increase virulence by destroying leukocytes and causing inflammation, but more evidence is needed to confirm this theory (Colin, Mazurier, Sire, & Finck-Barbancon, 1994; Kaneko, Kimura, Narita, Tomita, & Kamio, 1998; Meyer, Girardot, Piemont, Prevost, & Colin, 2009). Another key genetic variant in MRSA strains is the arginine catabolic mobile element (ACME). This element is unique to the community-associated MRSA strain USA300. The importance of the ACME is that it contains several *arc* genes that improve the fitness of the bacterium, which may result in strains that are more difficult to treat and control (Diep, Stone, et al., 2008).

Epidemiology of MRSA

When MRSA initially emerged, it was regarded as a nosocomial pathogen, which only infected people who had been exposed to healthcare facilities or had other risk factors such as drug abuse (David & Daum, 2010). This type of MRSA was later named healthcare-associated or healthcare-acquired MRSA (HA-MRSA). Several strains of HA-MRSA eventually spread across the globe with a strong prevalence in North America, South America, Europe and Asia (Diekema et al., 2001; Stefani et al., 2012). It is beneficial to note that the prevalence of HA-MRSA varies widely based on geographic locations, which researchers think is due to the success of prevention and eradication programs (Andersen, Rasch, & Syversen, 2007; Dulong, Haamann, Peters, Schablon, & Nienhaus, 2011; Kerttula et al., 2007; Moellering, 2012). Nevertheless, HA-MRSA quickly became a global health problem and remains one today.

The isolation of MRSA to healthcare facilities began to change in the 1990s, when MRSA infections were first identified in healthy people who lacked exposure to

healthcare facilities or other risk factors (David & Daum, 2010). Upon further analysis, it was discovered that new strains of MRSA had emerged. These strains were later named community-associated or community-acquired MRSA (CA-MRSA) due to their presence in healthy communities.

The first confirmed cases of CA-MRSA were identified from patients in Australia, who developed infections despite lacking exposure to healthcare facilities or other risk factors (Udo, Pearman, & Grubb, 1993). Soon after the outbreak in Australia, several children in the United States died from MRSA infections that were later identified as CA-MRSA ("Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*--Minnesota and North Dakota, 1997-1999," 1999). Infections caused by CA-MRSA strains continued to become more common, and today they account for the majority of MRSA infections. A recent study found that community-onset infections accounted for 90% of MRSA infections in San Francisco, CA (Liu et al., 2008). Another more recent study looked at the phenotypes of MRSA infections observed in U.S. hospitals between 2005 to 2008, and found that the proportion was 55% HA-MRSA, and 45% CA-MRSA, which remained constant throughout the three year study (Klein, Sun, Smith, & Laxminarayan, 2013).

There are several fundamental differences between strains of CA-MRSA and HA-MRSA, including the genetic makeup, associated risk factors, and prevalence. The main genetic differences between HA-MRSA and CA-MRSA concern the type of staphylococcal chromosomal cassette *mec* (*SCCmec*) incorporated into the genome. HA-MRSA strains carry the *mecA* gene on the larger *SCCmec* types I, II, or III (David & Daum, 2010). HA-

MRSA strains carrying these *SCCmec* types are often resistant to other classes of antibiotics in addition to the penicillins, cephalosporins and carbapenems. Additionally, HA-MRSA strains rarely carry the PVL gene (LaPlante, Rybak, Amjad, & Kaatz, 2007). The most common strains of HA-MRSA are USA100, USA800 and USA500 (Stefani et al., 2012).

In contrast, CA-MRSA strains carry the *mecA* gene on the smaller *SCCmec* type IV or V, which are less likely to encode multidrug resistance, a key difference between CA-MRSA and HA-MRSA (Ma et al., 2002). Additionally, many strains of CA-MRSA carry the PVL gene, which may enhance the virulence (LaPlante et al., 2007). The most commonly identified strains of CA-MRSA are USA300 and USA 400 (Stefani et al., 2012).

The risk factors associated with colonization or infection with HA-MRSA or CA-MRSA are quite different. The main risk factors associated with developing a HA-MRSA infection are exposure to healthcare facilities and suffering from a number of health problems (David & Daum, 2010). Additionally, older individuals are more likely to be hospitalized from HA-MRSA phenotypes (Klein et al., 2013). The most common types of HA-MRSA infections are pneumonia, bacteremia and invasive infection (Naimi et al., 2003). On the other hand, CA-MRSA infections usually appear in healthy people with no recent healthcare exposure (Herold et al., 1998). Additionally, CA-MRSA strains mainly cause skin and soft-tissue infections, seem to be more virulent and are more easily transmitted (DeLeo, Otto, Kreiswirth, & Chambers, 2010; Naimi et al., 2003). Several populations have been found to have a higher risk of becoming infected with CA-MRSA including prisoners (Maree et al., 2010), military personnel (Ellis, Hospenthal, Dooley,

Gray, & Murray, 2004), children in day care centers (Miller et al., 2011), and men who have sex with men (Diep, Chambers, et al., 2008). It is necessary to note that strains of CA-MRSA have become endemic in healthcare facilities, in addition to being present in the community (Maree, Daum, Boyle-Vavra, Matayoshi, & Miller, 2007).

Recently, attention has been given to the emergence of livestock-associated MRSA (LA-MRSA). *S. aureus* has the ability to infect many types of livestock including cows, pigs and chicken (Fitzgerald, 2012). This was not a serious concern until a recent report found MRSA isolates in livestock may have the potential to infect humans (Cuny et al., 2010). While LA-MRSA has not been identified as a major source of infections in humans, it is being studied because of the potential impact it could have.

Impact of MRSA

S. aureus is part of the natural flora in humans. It is commonly found on the skin, mucous membranes, axillae, vagina, pharynx and most frequently the nasal passage (Lowy, 1998; Noble, Valkenburg, & Wolters, 1967). Research has shown that about 25% to 30% of people are asymptomatically colonized with *S. aureus* and may be colonized for long periods of time (Gorwitz et al., 2008; Graham, Lin, & Larson, 2006; Kluytmans, van Belkum, & Verbrugh, 1997). However, of those people colonized with *S. aureus*, only about 0.8% to 1.5% had antimicrobial resistant strains.

People who become colonized with *S. aureus* often have no clinical symptoms or adverse health effects. Occasionally, under the right conditions, people who are exposed to *S. aureus* may develop an infection. In fact, *S. aureus* is the leading cause of infections across the globe (Diekema et al., 2001). Individuals colonized by *S. aureus*

have an increased risk of developing infections (von Eiff, Becker, Machka, Stammer, & Peters, 2001). The most common types of *S. aureus* infections include bloodstream, skin, soft tissue, bone, joint, and respiratory (Diekema et al., 2001). *S. aureus* can also cause several other types of adverse health effects, ranging from boils, cellulitis, wound infections, scalded skin syndrome, bacteremia, endocarditis, wound infections, to the less frequent pneumonia or toxic shock syndrome (Mahon & Manuselis, 1995). Additionally, *S. aureus* can produce an enterotoxin that can cause gastroenteritis if ingested (Lowy, 1998).

S. aureus has become an enormous problem in healthcare settings, where it is a leading cause of healthcare associated infections (HAIs). HAIs are infections that patients acquire while being treated for other conditions, and are thereby potentially preventable. The Centers for Disease Control and Prevention (CDC) has estimated that for every 20 people hospitalized in the U.S., one will develop an HAI (CDC, 2013). The most common types of HAIs include central line-associated bloodstream infections, ventilator-associated pneumonia, catheter-associated urinary tract infections and surgical site infections (Yokoe et al., 2008).

S. aureus infections were the most common cause of HAIs reported to the National Healthcare Safety Network (NHSN) in 2009-2010 (Sievert et al., 2013). This was an increase from 2006-2007 when they were the second most common cause (Hidron et al., 2008). However, only about 43-65% of the reported *S. aureus* HAIs were caused by methicillin-resistant strains, depending on the type of infection (Hidron et al., 2008; Sievert et al., 2013). Studies have also found that *S. aureus* HAIs were the most common

cause of surgical site infections and ventilator associated pneumonia (Hidron et al., 2008; Sievert et al., 2013)..

MRSA infections have a substantial impact on the United States population. In addition to HAIs, it was estimated that, in 2005, *S. aureus* infections caused about 478,000 hospitalizations, of which more than half (278,000) were related to MRSA (Klein, Smith, & Laxminarayan, 2007). In 2009, the estimated number of hospitalizations caused by *S. aureus* increased to 697,248, of which 463,017 hospitalizations were MRSA strains (Klein et al., 2013). Additionally, there are an estimated 94,360 invasive MRSA infections each year that result in about 18,650 deaths (Klevens et al., 2007). Up to 86% of invasive MRSA infections are HAIs, and potentially preventable.

The high percentage of *S. aureus* infections that are caused by MRSA strains is an important public health concern. MRSA infections are harder to treat and often have worse outcomes. MRSA infections are associated with increased morbidity and mortality when compared to methicillin susceptible strains of *S. aureus* (Cosgrove et al., 2003; Shorr et al., 2006).

Infections caused by MRSA also have an enormous economic impact around the globe. Annually, in the European Union, MRSA infections are estimated to cause one million extra days of hospitalization costing an extra €380 million (*ECDC/EMEA Joint Technical Report - The bacterial challenge: time to react* 2009). One U.S. study found that on average, a patient infected with a MRSA infection cost \$19,405 more to treat than a patient infected with susceptible *S. aureus* and were more likely to die (Filice et al., 2010). A breakdown of the extra costs associated with MRSA found that only 20% could

be attributed to increased infection control measures, such as improved contact precautions, antimicrobial therapy and laboratory measures (Goetghebeur, Landry, Han, & Vicente, 2007).

MRSA in Long-Term Care Facilities

The aging of the “baby boomer” generation in the U.S. is changing the makeup of the United States healthcare systems. The number of long-term care facilities (LTCF) is increasing, while the number of acute-care facilities (ACF) is decreasing (Manzur & Gudiol, 2009). This change is significant because the majority of MRSA related research has been focused on ACF, especially intensive care units.

There are several key characteristics of LTCF that increase the prevalence of MRSA infections. The population in LTCF is comprised of older individuals who often suffer from multiple health problems and have weakened immune systems (Richards, 2002). This makes them more susceptible to MRSA infections. Moreover, LTCF patients often suffer from conditions that impact their sanitary habits, which may increase the risk of acquiring infections. Additionally, LTCF serve as the patient’s residence and the patients are involved in group activities throughout the facility, such as physical therapy, meals, and social activities. This increases their exposure to other potentially infected individuals and contaminated surfaces (Smith et al., 2008). Research has demonstrated that the length of stay and multiple bed rooms are risk factors of MRSA transmission in LTCF (Wendt, Svoboda, Schmidt, Bock-Hensley, & von Baum, 2005).

Treatment of MRSA Infections

Currently, the number of effective antimicrobials used to treat MRSA infections

is dwindling. Treatment options can vary depending on the type of infection and other patient factors. Overall, the standard antimicrobial used to treat MRSA infections is vancomycin (Gould et al., 2012; Kumar & Chopra, 2013). Nevertheless, strains of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) have emerged (Hiramatsu et al., 1997). Due to this fact, the Infectious Disease Society of America (IDSA) recommends the use of other antimicrobials in situations where vancomycin may not be successful. These other antimicrobials include daptomycin, linezolid, clindamycin, telavancin, trimethoprim/sulfamethoxazole, and tetracycline, which can be used solo or in combinations depending on the type of infection, patient history, and other factors (Liu et al., 2011). Researchers are also working to develop new antimicrobials in hopes of keeping viable treatment options in the future. The IDSA has set the goal of developing 10 new antimicrobial drugs for multi-drug resistant organisms by 2020 ("The 10 x '20 Initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020," 2010).

Routes of Transmission

Preventing the transmission of MRSA in healthcare facilities is a prominent public health issue that could save thousands of lives. Overall, there are four main routes of transmission of MRSA in healthcare facilities. They are direct contact with infected or colonized individuals, indirect contact through contaminated hands of healthcare workers, contaminated air and contaminated environmental surfaces or equipment (Otter et al., 2011).

Direct contact transmission occurs when a person is exposed to MRSA by touching or coming into contact with an infected or colonized patient. This type of transmission could occur when susceptible patients are roomed with infected or colonized patients, but due to isolation and the use of contact precautions, this route is not seen as a major contributor to transmission.

The role of contaminated healthcare worker's hands is extremely important in the transmission of MRSA (Ludlam et al., 2010). The hands of healthcare workers can become directly contaminated while working with infected or colonized patients or indirectly by touching contaminated surfaces or equipment. If their hands are not decontaminated, they can spread MRSA to susceptible patients, who may then develop a new healthcare acquired infection. Additionally, contaminated hands can be a source of recontamination for environmental surfaces in other locations.

Contaminated air has also been implicated in the transmission of MRSA. This most often occurs when the MRSA infection affects the respiratory system (Sherertz et al., 1996). Air conditioning ducts have also been implicated (Wagenvoort, Davies, Westermann, Werink, & Toenbreker, 1993). Direct airborne transmission is not seen as a major contributor to new infections, but it has been shown to contribute to the contamination of environmental surfaces, which are a more effective route of transmission (Hardy, Oppenheim, Gossain, Gao, & Hawkey, 2006; Wilson et al., 2007).

Environmental surfaces contaminated with MRSA seem to be central for transmission in healthcare facilities. Surfaces can become contaminated through multiple paths: directly from bacteria shed by infected or colonized patients; indirectly

from contaminated hands of healthcare workers; and indirectly from airborne particles from patients or air ducts. Once these surfaces become contaminated with MRSA, they remain contaminated until the surfaces are cleaned or the MRSA dies, which could be up to a year (Kramer et al., 2006; Wagenvoort, Gelissen, & Timpert, 1997). This reinforces the importance of environmental cleaning programs.

Infection prevention programs have been developed to address each of these routes of transmission. Hand hygiene is the most powerful tool to reduce the contamination of healthcare workers hands. Active surveillance can be used to identify patients infected and colonized with MRSA. Once the patients are identified, healthcare workers can use the correct contact precautions such as gloves, gowns and masks when needed. Another tool is environmental cleaning. This project focused on the role of contaminated surfaces and equipment in the transmission of MRSA because the link between contaminated surfaces and the development of MRSA infections remains unclear and requires more research.

Environmental Decontamination

The basic understanding and approach for disinfecting patient-care items and equipment were developed over 40 years ago (Rutala & Weber, 2011). This approach uses three classifications for patient-care items and equipment. First are critical items that have a high risk of causing infections if contaminated. These are items inserted into sterile body sites and must be sterilized before use. Semi-critical items are used in contact with mucous membranes or non-intact skin and must undergo high levels of

disinfection. The last classification is non-critical items, which are items that may contact the intact skin and require low-level disinfection.

Environmental surfaces are considered non-critical items and, therefore, require low-level disinfection on a daily basis. A recent report by the CDC has outlined the guidelines for disinfection and sterilization in healthcare facilities (Rutala et al., 2008). This document emphasizes that while MRSA has developed resistance to several types of antibiotics, it can easily be eradicated through proper cleaning with detergents and disinfectants. They point out that cleaning solutions must be used at the correct concentrations, be applied to all surfaces, and remain in contact with the surfaces for an adequate time to kill the bacteria. The contact time for most cleaners is at least 10 minutes (Rutala et al., 2008). Nevertheless, these steps are not always followed, possibly leading to the persistence of viable MRSA on surfaces.

MRSA on Environmental Surfaces

Environmental surfaces can become contaminated with MRSA when bacteria are shed into the environment by infected or colonized people. This can occur during events such as sneezing, coughing, talking, eating, or routine medical care. Past studies have demonstrated that infected or colonized patients shed their specific strain of MRSA into the surrounding area, which results in high rates of contamination on surfaces and objects in proximity to the patient (Boyce et al., 1997; French et al., 2004; Sexton et al., 2006). Boyce, et al., (1997) observed more than 50% of floor samples, bed linen samples, and patient gown samples were positive for MRSA. Additionally, they found 85% of patients with MRSA infected wounds or urine had environmental MRSA

contamination compared to only 36% of patients with MRSA infection in the sputum, blood or conjunctivae. Contamination also increased with the number of culture positive body sites. Infected patients rooms were also found to have 32% of sampled surfaces contaminated compared to just 20% of surfaces in colonized patient rooms.

Once these bacteria are shed onto environmental surfaces, they remain there until they die or are removed by cleaning. If they are not cleaned or eradicated research has shown that MRSA may remain viable on environmental surfaces for long periods of time up to 318 days (Kramer et al., 2006; Wagenvoort et al., 1997; Wagenvoort, Sluijsmans, & Penders, 2000). There are a number of factors that impact the persistence of MRSA on environmental surfaces. Recent studies indicated lower temperatures, lower levels of humidity and the presence of organic material, such as bovine serum albumin (used to mimic organic material) all increased the length of survival (Coughenour, Stevens, & Stetzenbach, 2011; Noyce, Michels, & Keevil, 2006). Additionally, the type of materials surfaces are composed of impacts survival, with plastic and vinyl increasing survival times compared to wood (Coughenour et al., 2011). Incorporating copper into surfaces may also decrease survival times (Noyce et al., 2006). When comparing MRSA strains to non-resistant strains, survival times are similar (Neely & Maley, 2000), but MRSA strains that were more likely to cause an outbreak were shown to survive longer than normal MRSA strains (Wagenvoort et al., 2000).

The role of contaminated environmental surfaces in causing infections is still being debated. It is understood that the concentration of MRSA on environmental surfaces has been measured at levels high enough for transmission (Otter et al., 2011).

For example, a study found the average concentration of MRSA on surfaces to be between 1 Colony Forming Unit (CFU)/cm² to 100 CFU/cm² (Rutala, Katz, Sherertz, & Sarubbi, 1983), which is higher than the infectious dose of less than 15 *S. aureus* cells, shown to cause infection in experimental lesions (Foster & Hutt, 1960). Additionally, surfaces contaminated with MRSA were implicated in causing new infections during a MRSA outbreak in a London surgical ward (Jeanes, Rao, Osman, & Merrick, 2005). Furthermore, a study was able to match strain types of environmental samples and patients, implying 3 of 26 patients were infected from the environment (Hardy et al., 2006).

Many studies have been conducted to evaluate the presence of MRSA on a variety of surfaces, but the results have varied widely. Hardy et al., (2006) found MRSA present in 21.8% of samples at a hospital, with the area underneath the bed having the highest level of contamination. Boyce et al., (1997) had similar results when they examined surfaces in a hospital and found that 27% of environmental samples were MRSA positive, with the floor being the most contaminated. Sexton et al., (2006) examined a 720 bed hospital with two intensive care units and found much higher rates of contamination, with 56.3% of surface samples contaminated with MRSA. Door handles have also been found to be contaminated in 19% of MRSA patient rooms and 8.7% of all patient rooms (Oie, Hosokawa, & Kamiya, 2002).

Overall, the healthcare surfaces that are touched frequently and are in proximity of patients have been shown to have a higher frequency of contamination with MRSA (Boyce et al., 1997; Hayden, Blom, Lyle, Moore, & Weinstein, 2008; Huslage, Rutala,

Sickbert-Bennett, & Weber, 2010). These surfaces are called “high-touch surfaces” and include the bed rails, the bed surface, and the supply cart. Recently, environmental cleaning programs have been focusing cleaning efforts on these “high-touch” areas in the hope of reducing transmission, but more research is needed to evaluate the effectiveness. A recent study found that increasing the cleaning of “high touch” surface to twice daily reduced MRSA surface contamination from 14.6% to 9.1%. Additionally, it found that contamination on doctors and nurses hands by MRSA was reduced during the intervention (Wilson et al., 2011).

Environmental sampling has also been used to evaluate the presence of MRSA after several other types of cleaning interventions. One study evaluated the effect of adding an additional cleaner into a UK hospital and found that microbial contamination was lowered by 32.5%, which reduced new MRSA infections by 26% (Dancer, White, Lamb, Girvan, & Robertson, 2009). Disinfection wipes were found to reduce MRSA contamination on bed rails from 4.4 CFU/cm² to 0.4 CFU/cm² (Cheng, Boost, & Chung, 2011). A study of 10 intensive care units during enhanced cleaning intervention found that new MRSA infections were lowered from 3% to 1.5% (Datta, Platt, Yokoe, & Huang, 2011). They also found that patients who were placed in rooms previously occupied by a MRSA patient had an increased risk of developing an infection in the control group but not the intervention group. Another study found that 16% of sites contained MRSA after bleach and steam cleaning (Jeanes et al., 2005).

A newer type of disinfectant that has been shown to be highly effective in removing MRSA from the environment is hydrogen peroxide vapor (HPV). French et al.,

(2004) compared MRSA contamination following normal terminal cleaning procedures with the use of HPV. They found that before cleaning, 74% of swabs were positive for MRSA. After terminal cleaning, this was lowered to 66% of swabs being positive compared to only 1.2% positive swabs obtained after the use of HPV. Another study also found HPV was effective in removing MRSA from environmental surfaces in an intensive care unit, but once MRSA infected patients were readmitted the room quickly became recontaminated (Hardy et al., 2007). These two studies show HPV may be more effective than normal terminal cleaning, but more research is needed to determine the feasibility in multiple types of facilities.

MRSA contamination is not limited to rooms of infected patients. MRSA was identified in 43% of beds used by uninfected patients (Boyce et al., 1997). This contamination of uninfected rooms could be due to bacteria shed by previous occupants (French et al., 2004; Hardy et al., 2006), or recontamination from healthcare workers, visitors, or other patients (Otter et al., 2011). Healthcare workers are the most likely source of recontamination. A recent study found that compliance by healthcare workers to hand hygiene varies widely, with an overall average of only 40% (Boyce & Pittet, 2002). Furthermore, a study found that 66% of healthcare workers who work directly with MRSA patients obtain the patient's strain on their apron or gloves, and even if the healthcare worker did not have contact with the patient, 40% still became contaminated with MRSA (Boyce et al., 1997).

Cleaning of environmental surfaces and equipment is an essential tool in infection prevention, but it is often overlooked because surface contamination is not

thought to be a leading contributor to the transmission MRSA. Nevertheless, research has shown the hospital environment is not adequately cleaned. A recent study examined the thoroughness of terminal cleaning at 23 acute care hospitals and found that only 49% of surfaces were adequately cleaned (Carling, Parry, & Von Beheren, 2008). The study also identified the areas least likely to be cleaned were the toilet handholds, bedpans, light switches and door knobs, and most likely to be cleaned were the sinks, toilet seats and tray tables. This is particularly concerning because MRSA has been identified on hospital surfaces where it may directly or indirectly be transmitted to susceptible patients resulting in new infections.

This is a concern because MRSA has been identified on hospital surfaces where it may directly or indirectly be transmitted to susceptible patients, resulting in new infections. The majority of previous studies examining the presence of MRSA on surfaces have been conducted in acute care hospitals. With the growing number of long-term care facilities, it is vital to identify the differences in surface contamination between these locations. Additionally, to date no studies have evaluated the presence of MRSA on environmental surfaces in healthcare facilities in southern Nevada and the antimicrobial susceptibility profiles of environmental *Staphylococcus aureus* isolates present in southern Nevada have not been adequately examined.

Objectives

The role of environmental surface contamination in the transmission of MRSA is still unclear. It is known that patients infected or colonized with MRSA shed bacteria into the environment. Additionally, it has been found that MRSA can survive for long

periods of time on hospital surfaces and in dust. These bacteria that are present on hospital surfaces may be transmitted to new patients resulting in new colonization or infections. Few studies have examined the effectiveness of environmental cleaning in removing MRSA from environmental surfaces. In addition, the prevalence of MRSA in healthcare facilities in southern Nevada is unknown.

The objectives of this study are to:

- (1) Determine the prevalence of MRSA from colonized or infected patient's rooms on five different environmental surfaces pre-and post-cleaning at an acute care hospital and a long-term care facility.
- (2) Evaluate the effectiveness of the environmental cleaning in removing MRSA contamination from five high touch surfaces at each facility.
- (3) Compare the results from the acute care hospital and the long-term care facility.
- (4) Identify the antimicrobial susceptibility profiles of MRSA isolates present in southern Nevada.

Research Questions and Hypotheses

1. Is MRSA present on environmental surfaces in infected and colonized patient rooms pre-cleaning?
2. Do environmental cleaning programs effectively remove MRSA bacteria from environmental surfaces in infected or colonized patient rooms?

Hypothesis 1: The prevalence of MRSA will be higher in the pre-cleaning samples than the post-cleaning samples at the Acute Care Hospital.

H_{01} : There is no difference between the prevalence of MRSA in the pre-cleaning and post-cleaning samples collected at the Acute Care Hospital.

H_{A1} : There is a difference between the prevalence of MRSA in the pre-cleaning and post-cleaning samples collected at the Acute Care Hospital.

Hypothesis 2: The prevalence of MRSA in the pre-cleaning samples will be higher than the post-cleaning samples at the long-term care facility.

H_{02} : There is no difference between the prevalence of MRSA in the pre-cleaning and post-cleaning samples collected at the long-term care facility.

H_{A2} : There is a difference between the prevalence of MRSA in the pre-cleaning and post-cleaning samples collected at the long-term care facility.

CHAPTER 3

MATERIALS AND METHODS

Experimental Design

This study was conducted at an acute-care hospital (ACH) and a long-term care facility (LTCF) located in southern Nevada. Following the discharge of patients infected or colonized with MRSA, environmental surface samples were collected in patient rooms. In each patient room, 5 pre-terminal cleaning samples and 5 matching post-terminal cleaning samples were collected from five different environmental surfaces. Fifty swab samples were collected from five patient rooms at the ACH and the LTCF, and an additional 20 sponge samples were collected from two patient rooms at the LTCF, for a total of 120 samples. Following collection, the samples were analyzed for the presence of *S. aureus* and MRSA by culture analysis using two types of selective media. Any positive MRSA isolates underwent confirmatory analyses with real-time polymerase chain reaction (PCR) analysis for the *mecA* gene. To characterize each MRSA isolate, the antibiotic susceptibility profiles were identified using the VITEK® 2 Compact (bioMérieux, Durham, NC).

Quality Control Organisms and Culture Media

Four bacterial reference strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA). These included *Staphylococcus aureus* ATCC 25923, a methicillin-sensitive and coagulase positive strain, *Staphylococcus aureus* ATCC 43300 (MRSA) a methicillin-resistant strain, *Enterococcus faecalis* ATCC 29212, used for quality control of commercially prepared media and antimicrobial susceptibility testing, and

Staphylococcus aureus ATCC 29213, used as a standard for antimicrobial susceptibility testing. The media used for this study was obtained from BD Diagnostics (Sparks, MD) and included BBL™ CHROMagar™ *Staph aureus*, BBL™ CHROMagar™ MRSA, Difco™ tryptic soy agar, Trypticase™ soy agar with 5% sheep blood, and Trypticase™ soy broth with 6.5% sodium chloride (enrichment broth).

Sample Collection

Collection techniques and laboratory methods were adapted from previous work (Buttner et al., 2010; Ezeanolue, 2008). Environmental surface swabs were collected by trained hospital staff from each healthcare facility following standard surface sampling protocols (French et al., 2004). Following the discharge of patients infected or colonized with MRSA, 10 environmental surface samples (5 pre-cleaning and 5 post-cleaning) were collected in the room from five different environmental surfaces pre-terminal cleaning and then repeated at least one hour following post-terminal cleaning. The pre-cleaning and post-cleaning samples were collected from the same five locations in each patient room. The sampling locations included the floor, bed rail, TV remote control or call bell, bathroom doorknob, and bedside table (Figure 1). These locations were chosen because they are high-touch areas or were in proximity to the patients. The hospital staff identified an approximate 2" by 2" surface area on each surface to sample. The exact sampling locations were chosen by hospital staff to be as close to the patient bed as possible for the floor and bedside table and the area that was most likely to be touched by the hands of the patients for the bed rail, call bell, TV remote and bathroom

doorknob. Because all of the surfaces were different shapes and sizes, the area sampled was an approximation.

Two different sampling techniques were used. One hundred samples, fifty from each facility, were collected using a moistened sterile swab (BBL CultureSwab; BD Diagnostics) that was used to swab an approximate 2 inch by 2 inch surface area, in two directions, at each location and placed into a tube of sterile liquid Stuart transport medium (BD Diagnostics). An additional 20 samples were collected from the LTCF using sterile all-purpose sponges (Tyco Healthcare Group; Mansfield, MA) moistened with ultrapure water. The sponges were used as an alternative sampling method because of low positive results obtained using the swabs. The all-purpose sponge was used to wipe a 2 inch by 2 inch surface area, in two directions, and placed into a sterile tube of 10ml ultrapure water. All samples were refrigerated overnight at the healthcare facility, transported to the Emerging Diseases Laboratory, (University of Nevada, Las Vegas) the following day, and processed within 24 hours of collection.

Sample Processing

Sample analysis procedures were adapted from previous studies (Buttner et al., 2010; Ezeanolue, 2008). All swabs were streaked for isolation onto CHROMagar™ *Staph aureus* and CHROMagar™ MRSA (BD Diagnostics) and incubated in ambient atmosphere at 35°C for 24 hours. The swabs were then placed in enrichment broth (BBL Trypticase Soy Broth with 6.5% Sodium Chloride, BD Diagnostics), vortexed for 10 seconds and shaken at 130 rpm for 24 hours in a 35°C environmental shaker incubator. The incubation in enrichment broth was conducted to enhance the survival of stressed or

damaged bacteria on environmental surfaces and increase the ability to detect MRSA.

Following incubation, using a sterile loop, 10µl of the enrichment broth was subcultured onto CHROMagar™ *Staph aureus* and CHROMagar™ MRSA and streaked for isolation, followed by incubation in ambient atmosphere at 35°C for 24 hours.

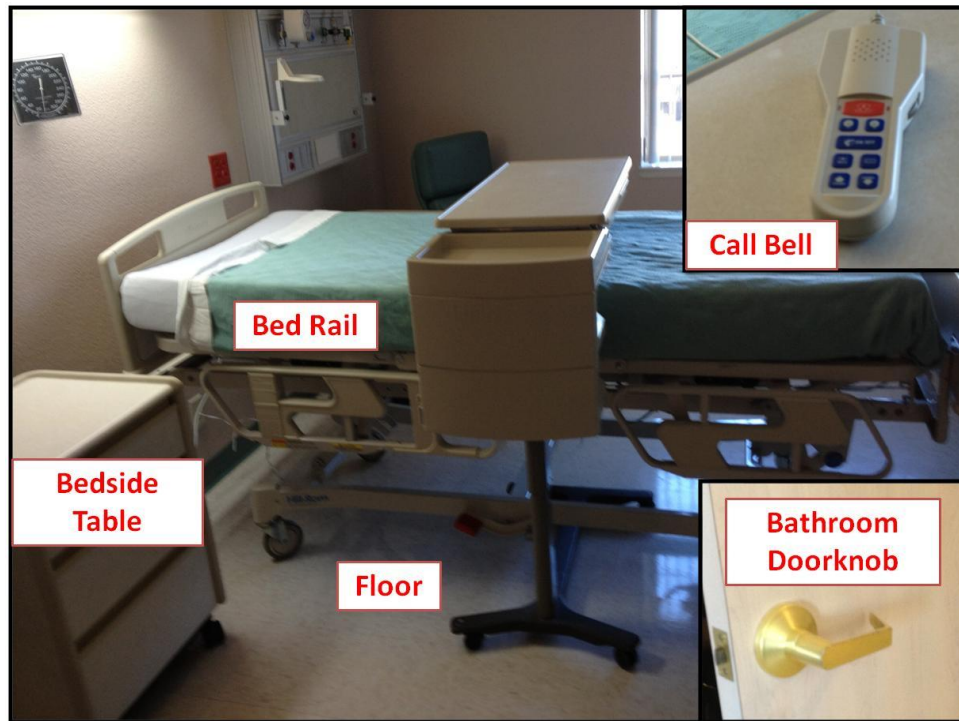


Figure 1. Environmental Sampling Locations. The five environmental sampling locations included the floor, bedside table, bed rail, call bell or television remote (not shown) and bathroom doorknob.

All sponge samples were vortexed in 10ml of ultrapure water for one minute and 100µl was spread plated onto CHROMagar™ *Staph aureus* and CHROMagar™ MRSA (BD Diagnostics) and incubated in ambient atmosphere at 35°C for 24 hours. Additionally, 100µl of the vortexed solution was dispensed into enrichment broth (BBL Trypticase Soy Broth with 6.5% Sodium Chloride, BD Diagnostics), vortexed for 10 seconds and shaken

at 130 rpm for 24 hours in a 35°C environmental shaker incubator. Following incubation, using a sterile loop, 10µl of the enrichment broth was subcultured onto CHROMagar™ *Staph aureus* and CHROMagar™ MRSA and streaked for isolation, followed by incubation in ambient atmosphere at 35°C for 24 hours.

All agar plates were inspected for positive colonies. Any CHROMagar™ MRSA plates without typical MRSA colonies after 24 hours of incubation were incubated for an additional 24 hours. All CHROMagar™ *Staph aureus* plates without typical staphylococcal colonies after 24 hours of incubation were discarded. Positive results were determined using the manufacturer's instructions. Mauve to orange/mauve colonies that grew on CHROMagar™ *Staph aureus* medium were identified as *S. aureus* isolates. Smooth, moderately sized mauve colonies that grew on CHROMagar™ MRSA medium at 24 hours were recorded as MRSA. Any mauve colonies that first appeared at 48 hours were confirmed with further testing. All presumptive MRSA isolates were then stored at -70°C for use in additional testing.

DNA Extraction and Purification

Several colonies from each MRSA positive sample were suspended in 500µl of 0.01 M potassium phosphate buffer with 0.05% Tween 20, pH 7.0 (Sigma-Aldrich Co., St. Louis, MO). Additionally, 500µl of the liquid Stuart transport medium was collected from the post-cleaning samples corresponding to each of the MRSA positive pre-cleaning samples to test for the presence of DNA from non-viable MRSA. The DNA was extracted from the 500µl samples using an UltraClean Soil DNA Isolation Kit (MO BIO, Carlsbad, CA) following the manufacturer's protocol. The DNA was resuspended in 50 µl of Tris-

EDTA buffer pH 8.0 (Sigma-Aldrich, St. Louis, MO), diluted 10-and 100-fold, and stored at -70°C for future use.

Real-Time Polymerase Chain Reaction

A real-time PCR assay was conducted for all of the culture identified MRSA isolates and the corresponding culture negative post-cleaning samples to detect the *mecA* gene (P. Francois et al., 2003). The procedure used was adapted from previous studies (Buttner et al., 2010; Ezeanolue, 2008). The analysis was performed with the 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using the TaqMan® technology (Applied Biosystems). The probes and primers were the same as used in previous studies (P. Francois et al., 2003). The sequence of the forward primer was 5'-CATTGATCGCAACGTTCAATTT-3', the reverse primer was 5'-TGGTCTTTCTGCATTCTGGA-3' and the probe was 5'-6FAM-TGGAAGTTAGATTGGGATCATAGCGTCAT-TAMRA 3'. All DNA samples were analyzed at three different concentrations (undiluted, 10^{-1} and 10^{-2}) to avoid false negative results. Each PCR reaction contained a total volume of 25µl, that included nuclease free water (Promega, Madison, WI), 1X of TaqMan® Universal PCR Master Mix (Applied Biosystems), 0.1µM of the *mecA* forward primer (Eurofins MWG Operon, Huntsville, AL), 0.1µM of the *mecA* reverse primer (Eurofins MWG Operon), 0.075µM of the *mecA* probe (ABI) and 5µl of template DNA. The instrument was operated in standard mode with the following parameters: 2 min at 50°C , 10 min at 95°C , and 40 cycles of 15 sec at 95°C followed by 1 min at 60°C . All samples were amplified in duplicate and a non-template control (nuclease free water) and a positive control (ATCC 43300, a MRSA strain) were included

in each analysis. A TaqMan® exogenous internal positive control using a VIC™ probe (Applied Biosystems) was also used to test for inhibition in selected samples. Following completion of amplification, the results from each run were analyzed using the AB software Sequence Detection System (SDS) version 2.3. Amplification was reported by the mean C_T value of two replicates. The definition of a C_T value is the PCR cycle number when a detectable amplification product crosses the threshold.

Antimicrobial Susceptibility Testing

All positive MRSA isolates were subjected to antibiotic susceptibility testing (AST) using the gram positive *S. aureus* specific AST-GP71 card for the VITEK® 2 Compact (bioMérieux, Durham, NC) following the manufacturer's protocol. The concentrations of the antimicrobial agents analyzed are shown in Table 1. Briefly, the MRSA isolates and quality control (QC) organisms (*E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213) were subcultured onto Trypticase® Soy Agar with 5% Sheep Blood (TSAB, BD Diagnostics) in a Bio Safety Cabinet and incubated for 18-24 hours in ambient atmosphere at 35°C. Several colonies were picked using sterile cotton swabs and suspended into 3.0ml of sterile 0.45% sodium chloride inhalation solution (Care Fusion, Yorba Linda, CA) until an optical density of 0.5 – 0.63 McFarland standard was observed. Next 285µl of this solution was resuspended into 3.0ml of sterile 0.45% sodium chloride solution to be used for analysis. The MRSA and QC suspensions were loaded onto the AST-GP71 cards, and then analyzed in the VITEK® 2 Compact. Results of the susceptibility profile were stored electronically in the system and evaluated.

Table 1. Antimicrobial Agents analyzed by the VITEK® 2 Compact AST-GP71 Card.

Antimicrobial Agent	Concentration(s) µg/ml
Benzylpenicillin	0.125, 0.25, 1
Beta-Lactamase	N/A*
Cefoxitin Screen	6
Ciprofloxacin	1, 2, 4
Clindamycin	0.5, 1, 2
Daptomycin	0.5, 1, 2, 4, 16
Erythromycin	0.25, 0.5, 2
Gentamicin	8, 16, 64
Inducible Clindamycin Resistance	CM 0.5, CM/E 0.25/0.5*
Levofloxacin	0.25, 2, 8
Linezolid	0.5, 1, 2
Minocycline	0.12, 0.5, 1
Moxifloxacin	0.25, 2, 8
Nitrofurantoin	16, 32, 64
Oxacillin	0.5, 1, 2
Quinupristin/ Dalfopristin	0.25, 0.5, 2
Rifampicin	0.25, 0.5, 2
Tetracycline	0.5, 1, 2
Tigecycline	0.25, 0.5, 1
Trimethoprim/ Sulfamethoxazole	2/38, 8/152, 16/304
Vancomycin	1, 2, 4, 8, 16
*N/A = not applicable; CM = Clindamycin; E = Erythromycin	

Statistical Analysis

A total of 120 samples were collected in this study. The results were analyzed to determine the prevalence of MRSA pre-cleaning and post-cleaning. A Wilcoxon signed-rank test was performed to compare the prevalence of MRSA in pre-cleaning versus post-cleaning results at each facility. This test was chosen because it is a non-parametric statistical test used to compare pre- and post-paired samples and is valid for small sample sizes. The Wilcoxon signed-rank test, descriptive and inferential statistics were calculated using SPSS version 19.0.

CHAPTER 4

RESULTS

Environmental Sample Analysis

A total of 120 environmental surface samples were obtained from the two healthcare facilities; 50 from the acute care hospital (ACH) and 70 from the long-term care facility (LTCF). The detection limit for the 2" by 2" surface sampling area was estimated to be 0.4 CFU/cm². Culture analysis followed by confirmatory PCR analysis for the *mecA* gene identified that 18 of the 120 samples (15%) contained viable MRSA. Sixteen of the 18 MRSA positive samples were obtained 48 hours after direct inoculation onto CHROMagarTM MRSA media. Two additional MRSA positive samples were obtained after 24 hour incubation in enrichment broth followed by inoculation onto CHROMagarTM MRSA media. Of these two samples, one was obtained 24 hours after inoculation and one was obtained 48 hours after inoculation.

The distribution of the positive samples by collection time, facility and sampling technique can be seen in Table 2. Of the 50 samples collected at the ACH, 3 samples (6%) were MRSA positive compared to 15 samples (21%) of the 70 samples collected at the LTCF. Of the total 18 MRSA positive samples, 16 samples (89%) were collected pre-terminal cleaning, and 2 samples (11%) were collected post-terminal cleaning. A distribution of the total samples collected and the number and percent of MRSA positive samples by location and collection time can be seen in Table 3. Overall, the call bell had the highest percentage of MRSA positive samples followed by the floor and bed rail and the bedside table had the lowest.

Table 2. The collection time, facility and sampling method of the 18 MRSA culture positive environmental samples				
	Pre-Terminal Cleaning		Post-Terminal Cleaning	
Total Swab Samples	Samples collected	MRSA Positive	Samples collected	MRSA Positive
Acute Care Hospital	25	3 (12%)	25	0 (0%)
Long-Term Care Facility	25	9 (36%)	25	2 (8%)
Total Sponge Samples				
Long-Term Care Facility	10	4 (40%)	10	0 (0%)
Total Samples	60	16 (26.7%)	60	2 (3.3%)

Further analysis of the MRSA positive environmental samples showed that of the 12 rooms sampled, 7 rooms (58%) had a MRSA positive sample from at least one location and 5 rooms (42%) did not have any MRSA positive samples. Additionally, 4 rooms (33%) had MRSA positive samples from more than one location. Analysis of the sample collection time showed 6 rooms (50%) had pre-cleaning MRSA positive samples, while only 1 room (8%) had post-cleaning positive samples.

A Wilcoxon signed-rank test was performed, using SPSS 19.0, to evaluate the difference in MRSA prevalence in pre-cleaning compared to post-cleaning samples at the ACH and LTCF. The results showed there was not a statistically significant difference at the $\alpha = 0.05$ level between the prevalence of MRSA positive pre-cleaning samples and post-cleaning samples collected from the five different environmental surfaces at the acute care hospital ($Z = -1.732$, $p = 0.083$), but there was a statistically significant difference between the prevalence of MRSA positive pre-cleaning samples and post-

cleaning samples collected from the five different environmental surfaces at the long-term care facility ($Z = -3.317$, $p = 0.001$). The sample size for each surface was under 20 and therefore inadequate to statistically analyze the differences between surfaces.

Table 3. Number of total samples and MRSA culture positive samples by collection time and location						
Collection Time	Pre-Terminal Cleaning		Post-Terminal Cleaning		Total	
Surface	Samples collected	MRSA Positive	Samples collected	MRSA Positive	Samples collected	MRSA Positive
Floor	12	5 (42%)	12	1 (8%)	24	6 (25%)
Call Bell	7	4 (57%)	7	1 (14%)	14	5 (36%)
Bathroom doorknob	12	2 (17%)	12	0 (0%)	24	2 (8%)
Bed Rail	12	4 (33%)	12	0 (0%)	24	4 (17%)
Bedside Table	12	0 (0%)	12	0 (0%)	24	0 (0%)
TV Remote	5	1 (20%)	5	0 (0%)	10	1 (10%)
Total	60	16 (27%)	60	2 (3%)	120	18 (15%)

PCR Results

The C_T values for each of the 18 MRSA isolates can be seen in Table 4. Each sample was analyzed at three concentrations (undiluted, 10^{-1} and 10^{-2}), but the results for the undiluted samples were negative due to inhibition of the PCR reaction. Selected undiluted samples were tested with an internal positive control (IPC) to confirm inhibition. The IPC C_T value was 29.64 and all of the undiluted samples tested had IPC values at least 1 C_T value higher, indicating inhibition (see Appendix A for results). Isolates were considered positive for the *mecA* gene if the corresponding C_T value was <40 . Additional real-time PCR testing was conducted on the corresponding post-

cleaning environmental samples for each of the MRSA culture positive samples. All of the samples had an undetermined C_T value, indicating the *mecA* gene was not detected in rooms post-cleaning (Appendix B).

Table 4. Real time PCR analysis of environmental samples for the presence of the *mecA* gene at two concentrations.

Facility	Sample ID	Sample Location	Pre or Post-cleaning	Mean C_T value	
				DNA Dilution	
				10^{-1}	10^{-2}
ACH	4B	Bed Rail	Pre	22.2	25.2
ACH	4C	TV Remote	Pre	21.6	25.3
ACH	5A	Floor	Pre	22.4	25.4
LTCF	6A	Floor	Pre	29.0	31.9
LTCF	6B	Bed Rail	Pre	26.3	30.0
LTCF	6C	Call Bell	Pre	28.6	31.6
LTCF	6D	Bathroom doorknob	Pre	28.3	31.8
LTCF	6F	Floor	Post	25.7	29.7
LTCF	6H	Call Bell	Post	26.5	30.2
LTCF	7A	Floor	Pre	27.1	30.6
LTCF	7B	Bed Rail	Pre	32.9	36.5
LTCF	7C	Call Bell	Pre	24.5	28.2
LTCF	7D	Bathroom doorknob	Pre	25.1	28.7
LTCF	8C	Call Bell	Pre	25.7	29.6
LTCF	11A	Floor	Pre	20.1	23.1
LTCF	11B	Bed Rail	Pre	22.0	24.9
LTCF	11C	Call Bell	Pre	25.7	26.8
LTCF	12A	Floor	Pre	31.4	34.9

Antibiotic Susceptibility

The antimicrobial susceptibility profile was obtained with the VITEK® 2 Compact

for each of the 18 MRSA isolates (Appendix C). Overall, there were 16 different antimicrobial susceptibility profiles from the 18 isolates. The antimicrobial susceptibility profiles for the pre- and post-terminal cleaning MRSA isolates collected from the same location were found to have several differences when compared (Table 5).

Table 5. Comparison of the antimicrobial susceptibility profiles of the pre-cleaning versus post-cleaning samples from the same location in the same patient room.

Collection time	Pre-Cleaning	Post-Cleaning		Pre-Cleaning	Post-Cleaning
Sample	6A	6F		6B	6H
Location	Floor	Floor		Call Bell	Call Bell
Antimicrobial Agent					
Beta-Lactamase	+	+		+	+
Cefoxitin Screen	+	+		+	+
Benzylpenicillin	R	R		R	R
Oxacillin	R	R		R	R
Gentamicin	S	S		S	S
Ciprofloxacin	R	R		R	R
Levofloxacin	R	R		R	R
Moxifloxacin	I	R		I	I
Inducible Clindamycin Resistance	-	+		-	+
Erythromycin	R	R		S	R
Clindamycin	R	R		S	R
Quinupristin/Dalfopristin	S	S		S	S
Linezolid	S	S		S	S
Daptomycin	S	S		S	S
Vancomycin	S	S		S	S
Minocycline	S	S		S	S
Tetracycline	S	S		S	S
Tigecycline	S	S		S	S
Nitrofurantoin	S	S		S	S
Rifampicin	S	S		S	S
Trimethoprim/Sulfamethoxazole	R	S		R	R

S = susceptible; R = resistant; I = intermediate susceptibility; - represents negative result; + represents positive result

The percentage of total isolates susceptible to each of the antimicrobial agents tested is shown in Figure 2. All of the isolates were susceptible to daptomycin, linezolid, minocycline, quinupristin/dalfopristin, rifampicin, and tigecycline. Additionally, all of the isolates were resistant or intermediate to benzylpenicillin, ciprofloxacin, levofloxacin, moxifloxacin and oxacillin. All isolates had positive results for Beta-lactamase and ceftioxin screen. Only one isolate was not susceptible to vancomycin. The susceptibility to the remaining antimicrobials varied among the isolates.

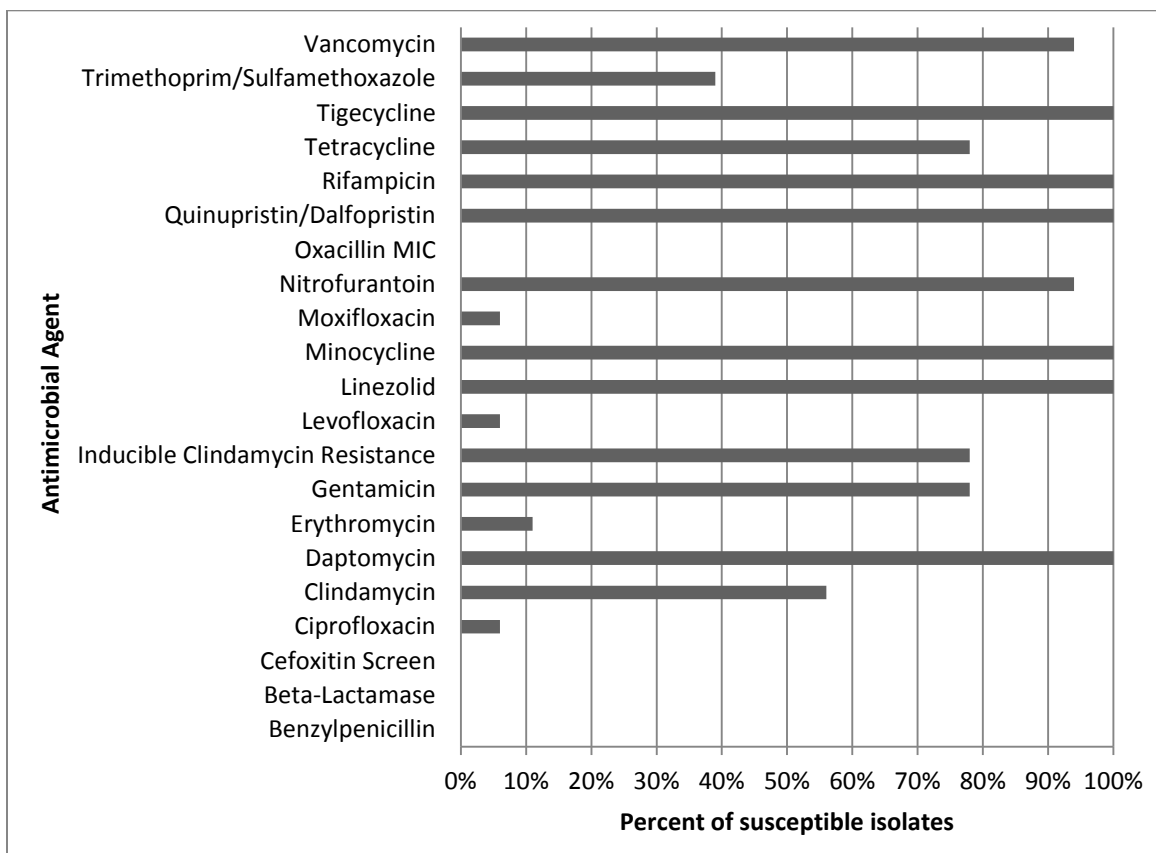


Figure 2. The percentage of MRSA isolates susceptible to antimicrobial agents. The antimicrobial susceptibility profiles of the 18 environmental MRSA isolates collected at two healthcare facilities in southern Nevada. Isolates were analyzed by the VITEK® 2 Compact using the AST-GP71 card (bioMérieux, Durham, NC).

CHAPTER 5

DISCUSSION

MRSA Prevalence

The main objective of this study was to determine the prevalence of MRSA from colonized or infected patients' rooms on five different environmental surfaces pre-terminal and post-terminal cleaning at two healthcare facilities. Overall, the percent of total samples positive for MRSA collected at both facilities was 27% for pre-terminal cleaning samples, 3% for post-terminal cleaning samples, and 15% for total samples collected. Previous environmental sampling studies have had varying results, with the percent of MRSA positive samples detected ranging from 10% (Rampling et al., 2001) to 61% (Chang et al., 2009). The results of this study are within that range, but are closer to the lower prevalence results that have been seen.

Facility Differences

The results differed between the acute care hospital (ACH) and the long-term care facility (LTCF). The prevalence of MRSA was found to be statistically significant between the pre-cleaning and post-cleaning samples collected at the LTCF ($p=0.001$) but not at the ACH ($p = 0.083$). The ACH had samples positive for MRSA in 12% of pre-terminal cleaning, 0% of post-terminal cleaning and 6% of total collected, which is considerably lower than previous studies conducted in hospitals (Blythe, Keenlyside, Dawson, & Galloway, 1998; Boyce et al., 1997; Chang et al., 2009; French et al., 2004; Hardy et al., 2006; Rampling et al., 2001; Sexton et al., 2006; Wilson et al., 2011). In contrast, the LTCF had 43% of pre-terminal cleaning, 6% of post-terminal cleaning and

21% of total collected samples positive for MRSA. This is comparable with the previous studies conducted in acute care hospitals, but to our knowledge no comparable studies have been conducted in LTCF.

The percentage of MRSA-positive environmental samples has been shown to have a high variability, which may be dependent on many factors. One key factor is the status of the patient who occupied the room prior to sampling. Colonized patients have been found to shed fewer bacteria than infected patients, and the amount shed by infected patients can vary depending on the type of infection (Boyce et al., 1997). This study did not differentiate between colonized and infected patients, and including both may have resulted in a lower positivity rate. Additionally, no health information was collected on the patients' status.

Results can also vary depending on the sampling technique used (Galvin, Dolan, Cahill, Daniels, & Humphreys, 2012). This study used a swab sampling technique comparable to many previous studies (Blythe et al., 1998; Boyce et al., 1997; Chang et al., 2009; Hardy et al., 2006; Rampling et al., 2001). One factor that may have impacted the results was the use of swabs originally designed to be used as a nasal swab and not a swab specifically designed for environmental surfaces. These swabs had a small surface area and could only be used to sample approximately 2" by 2" on each surface. In addition, each surface had a different total surface area and the proportion of total surface area sampled differed for each surface.

Prevalence of MRSA Post-terminal Cleaning

The percent of MRSA positive post-terminal cleaning samples observed in this

study is lower than those observed in previous studies (Blythe et al., 1998; French et al., 2004; Hardy et al., 2007; Wilson et al., 2011). This was expected if the rooms were adequately cleaned. A study by French et al., (2004) observed a 66% MRSA positivity rate of post-cleaning samples collected in a surgical ward with a detergent sanitizer used for cleaning. Another study conducted in a surgical ward during an outbreak of MRSA found only 16% of post-cleaning samples positive for MRSA, but they used bleach and steam cleaning (Jeanes et al., 2005). The samples in this study were collected in a different setting, an acute care hospital and a long-term care facility, that serve different populations and may use different cleaning procedures, which may have resulted in lower prevalence rates. Both of the facilities in this study use quaternary ammonium disinfectants (Appendix D) approved by the Environmental Protection Agency (EPA) to be effective against MRSA (EPA, 2009). Additionally, the low prevalence of MRSA positive post-cleaning samples does not prove that the room was free from MRSA. The relatively small sampling area of the swabs may not have been able to detect viable MRSA present throughout the room.

Although cleaning was found to be effective, two viable MRSA isolates were collected after terminal cleaning. Both of these isolates were identified from the same patient room at the LTCF. There are two explanations for why these samples were detected post-cleaning. The first is that the MRSA shed by the infected or colonized patient was not removed or eradicated during the cleaning process. Environmental cleaning has been shown to be ineffective if the cleaning solutions are not used at the appropriate concentrations or not applied to all surfaces for the proper contact times

(Rutala et al., 2008). The second possibility is that the room could have been recontaminated after cleaning by the environmental cleaning staff or another healthcare worker (Otter et al., 2011). Upon further analysis of the pre-cleaning and post-cleaning samples that were collected from the same location in the same room, it was found that the isolates had different antibiotic susceptibility profiles, suggesting that the room may have been recontaminated (Table 4). Additional analyses, such as strain typing, would be required to confirm this difference.

Surface Differences

This study also examined the prevalence of MRSA on six different surfaces (floor, bedside table, bed rail, call bell, television remote, and bathroom doorknob). Each of these surfaces is composed of different materials. The floors were vinyl, a surface on which MRSA has been shown to have an increased survival time compared to wood and metal (Coughenour et al., 2011). This may have contributed to 6 out of the 18 MRSA positive samples being collected from the floor, which corroborates previous studies that have also found the floor to have a high level of contamination (Blythe et al., 1998; Boyce et al., 1997; Hardy et al., 2006).

Several of the other surfaces consisted of plastic, which has been shown to increase MRSA survival time (Coughenour et al., 2011). The plastic bed rails accounted for 4 of the 18 MRSA positive samples. The television remote and call bell were also made of plastic and selected because they are frequently touched by the infected or colonized patient. The television remote only accounted for 1 of the 18 MRSA positive samples. On the other hand, the call bell accounted for 5 of the 18 MRSA positive

samples. This was the most frequently contaminated surface, which confirms previous research that showed high touch surfaces are more likely to be contaminated (Huslage et al., 2010).

The bathroom doorknob was made of metal and only accounted for 2 of the 18 MRSA positive samples. A study by Oie et al., (2002) sampled door handles in a Japanese hospital and found 19% of MRSA patient room door handles contained viable MRSA. This study found a similar rate of pre-cleaning samples of the bathroom doorknobs with 17% positive.

The last surface was the bedside table, which did not produce any MRSA positive samples. There are several possible explanations for this observation. The bedside tables consisted of a composite wood and plastic material. The surface of this material could be difficult to collect MRSA from, or perhaps survival was low on this material. Additionally, this surface could be easier to clean or was more thoroughly cleaned by staff.

Antimicrobial Susceptibility Profiles

One of the first studies to analyze the antimicrobial susceptibility profiles of MRSA isolates was the SENTRY Antimicrobial Surveillance Program, which collected data from 1997-1999, in the United States, Canada, Latin America, Europe, and the Western Pacific region (Diekema et al., 2001). This study found the susceptibility rates of MRSA isolates collected in the U.S. to be 64.5% for gentamicin, 96.3% for rifampicin, 11.4% for ciprofloxacin, 84.6% for tetracycline, 20.8% for clindamycin, 6.3% for erythromycin, and 74.0% for trimethoprim-sulfamethoxazole (TMP-SMZ). Our study found comparable

results for several of the antimicrobials including gentamicin (78%), rifampicin (100%), ciprofloxacin (6%), tetracycline (78%), and erythromycin (11%). One difference observed was the susceptibility rate of 39% for TMP-SMZ, which was much lower in our study than rates in the U.S. (74.0%) and much more comparable to the rates seen in Latin America (34.6%).

Another study analyzed the susceptibility of CA-MRSA strains to distinguish them from HA-MRSA strains. Isolates were analyzed from patients with MRSA respiratory infections, and the following susceptibility rates were found: vancomycin (100%), gentamicin (86%), clindamycin (39%), quinolones (49%), and erythromycin (12%) (Almer et al., 2002). Our results were similar for vancomycin (94%), gentamicin (78%), clindamycin (56%), quinolones (44%), and erythromycin (11%) suggesting a majority of our isolates may be CA-MRSA strains, but this must be confirmed by strain typing. This is important information because CA-MRSA strains are usually more virulent and have worse outcomes than HA-MRSA strains.

Study Limitations

There are several limitations to this study. The first limitation is only a relatively small surface area in each room was sampled, which may have resulted in not identifying all of the contamination. Second, the sampling method used may not have been the most effective available. A recent paper found contact plates are more efficient than swabs for recovering adsorbed cells (Obee, Griffith, Cooper, & Bennion, 2007). Third, there are no recognized environmental sampling standards making it difficult to compare results to other publications. Fourth, the sample size (120 total

samples) was small and only included two facilities. Lastly, this study included both infected and colonized patients but did not differentiate between the two. Previous studies have found colonized patients shed more bacteria into the environment (Boyce et al., 1997), which could have impacted the results of this study.

CHAPTER 6

CONCLUSION

The discovery of antibiotics has drastically reduced the number of people who die from bacterial infections each year. However, the emergence of antibiotic resistant bacteria continues to threaten the ability to treat these infections. Recently, antibiotic resistant pathogens have been emerging in community settings, which may increase the impact they have on populations. Additionally, several pathogens have become resistant to multiple types of antimicrobials, making treatment more difficult.

One of the most concerning antimicrobial resistant bacteria is MRSA. Since it emerged, it has spread throughout the world and now is present in both the community and healthcare facilities. MRSA remains a leading cause of healthcare acquired infections and results in about 460,000 hospitalizations and 19,000 deaths in the United States each year (Klein et al., 2013; Klevens et al., 2007). This is an unacceptable burden and a serious public health problem that requires solutions.

Contaminated environmental surfaces are a public health concern because, if these surfaces are not properly cleaned, they become a potential source of transmission of diseases such as MRSA. The current literature has demonstrated MRSA is present on environmental surfaces in healthcare facilities where it can survive for long periods of time and potentially cause infections. This study confirmed previous studies and provided information on the prevalence of MRSA on environmental surfaces at an acute care hospital and long-term care facility in southern Nevada.

This study was designed to determine the prevalence of MRSA on five different environmental surfaces pre and post terminal cleaning at an acute care hospital and a long-term care facility. The results demonstrated that viable MRSA was present on surfaces at both facilities. At the ACH, MRSA was present in 12% of pre-terminal cleaning, and 0% of post-terminal cleaning samples. At the LTCF, MRSA was present in 43% of pre-terminal cleaning samples and 6% of post-terminal cleaning samples. The location of the positive pre-cleaning samples included the floor (5), call bell (4), bed rail (4), bathroom doorknob (2) and TV remote (1), and the positive post-cleaning samples included the floor (1) and call bell (1). The floor, call bell, and bed rail may have been more likely to be contaminated with MRSA because of the survival of MRSA on the surface material and the proximity to the patient. Overall, the cleaning programs were effective and only two post-cleaning samples yielded viable MRSA, which were most likely caused by recontamination. The antibiotic susceptibility profile of the isolates obtained in the study varied widely, but the majority (94%) was susceptible to vancomycin, which is the main antibiotic used to treat MRSA infections.

The results of this study have several implications for future research. Overall, the results suggested long-term care facilities can be contaminated with MRSA pre and post-cleaning. Therefore, more resources should be used to further investigate long-term care facilities. Identifying the strain types for each of the isolates identified in this study would also be useful. This would help identify which strains are present in each facility and could be compared to other strains around the country and globe. In addition, resources should be used to identify the most effective environmental

cleaning programs and education programs for staff. Finally, future work should be focused on establishing environmental sampling standards for healthcare facilities. Currently, environmental sampling is rarely used in healthcare facilities unless there is an outbreak. Nevertheless, it is nearly impossible to determine if a room is clean with the naked eye because microorganisms can't be seen and a room that looks clean may be contaminated. It is clear that there is room for improvement in the cleaning of healthcare facilities. One way to do this would be to conduct daily environmental sampling, using large area surface sampling methods, of high touch surfaces, such as the bed rail, floor and call bell in patient rooms. These samples could be analyzed for antibiotic resistant bacteria, such as MRSA, to ensure that rooms are adequately cleaned. Implementing environmental monitoring in all healthcare facilities would help improve cleaning programs and reduce transmission of bacteria such as MRSA.

APPENDIX A. INTERNAL POSITIVE CONTROL PCR RESULTS

IPC Results

Real time PCR analysis of environmental samples for the presence of the *mecA* gene.

Facility	Sample ID	Sample Location	Pre or Post-cleaning	Mean C _T value	
				IPC	mecA
ACH	4G	Bed Rail	Pre	30.55	UD
ACH	4H	TV Remote	Pre	30.55	UD
ACH	5F	Floor	Pre	34.34	UD
LTCF	6G	Bed Rail	Pre	32.54	UD
LTCF	6I	Bathroom doorknob	Pre	29.87	UD
LTCF	7F	Floor	Pre	30.10	UD
LTCF	7G	Bed Rail	Pre	30.21	UD
LTCF	7H	Call Bell	Pre	30.12	UD
LTCF	7I	Bathroom doorknob	Pre	30.17	UD
LTCF	8H	Call Bell	Pre	30.44	UD
LTCF	11F	Floor	Pre	UD	UD
LTCF	11G	Bed Rail	Pre	37.02	UD
LTCF	11H	Call Bell	Pre	38.93	UD
LTCF	12F	Floor	Pre	38.79	UD
	NTC			29.64	UD
	NAC			UD	UD
	MRSA +			UD	20.53

NTC = No template control

NAC = No Amplification Control

UD = Undetermined

Appendix B. Corresponding Post-Cleaning Sample PCR Results

Real time PCR analysis of environmental samples for the presence of the *mecA* gene at two concentrations.

Facility	Sample ID	Sample Location	Pre or Post-cleaning	Mean C _T value		
				DNA Concentration		
				Undiluted	10 ⁻¹	10 ⁻²
ACH	4G	Bed Rail	Pre	UD	UD	UD
ACH	4H	TV Remote	Pre	UD	UD	UD
ACH	5F	Floor	Pre	UD	UD	UD
LTCF	6G	Bed Rail	Pre	UD	UD	UD
LTCF	6I	Bathroom doorknob	Pre	UD	UD	UD
LTCF	7F	Floor	Pre	UD	UD	UD
LTCF	7G	Bed Rail	Pre	UD	UD	UD
LTCF	7H	Call Bell	Pre	UD	UD	UD
LTCF	7I	Bathroom doorknob	Pre	UD	UD	UD
LTCF	8H	Call Bell	Pre	UD	UD	UD
LTCF	11F	Floor	Pre	UD	UD	UD
LTCF	11G	Bed Rail	Pre	UD	UD	UD
LTCF	11H	Call Bell	Pre	UD	UD	UD
LTCF	12F	Floor	Pre	UD	UD	UD

UD = Undetermined

APPENDIX C. ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF ENVIRONMENTAL MRSA

ISOLATES

ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF ENVIRONMENTAL MRSA ISOLATES

Sample ID	4B	4C	5A	6A	6B	6C	6D	6F	6H	7A	7B	7C	7D	8C	11A	11B	11C	12A
Location	Bed rail	TV remote	Floor	Floor	Bed rail	Call Bell	Bathroom Doorknob	Floor	Bed Rail	Floor	Bed rail	Call bell	Bathroom Doorknob	Call bell	Floor	Bed rail	Call bell	Floor
Pre or post clean	Pre	Pre	Pre	Pre	Pre	Pre	Pre	Post	Post	Pre	Pre	Pre	Pre	Pre	Pre	Pre	Pre	Pre
Antimicrobial Agent																		
Beta-Lactamase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cefoxitin Screen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Benzylpenicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Oxacillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Gentamicin	S	S	I	S	S	S	S	S	S	I	S	S	I	R	S	S	S	S
Ciprofloxacin	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Levofloxacin	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Moxifloxacin	R	S	I	I	I	R	R	R	I	R	I	R	I	I	R	R	R	R
Inducible Clindamycin Resistance	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
Erythromycin	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Clindamycin	S	S	S	R	S	R	R	R	R	R	S	R	S	S	S	R	S	S
Quinupristin/Dalfopristin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Linezolid	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Daptomycin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Vancomycin	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S
Minocycline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Tetracycline	S	S	S	S	S	R	R	S	S	S	S	R	S	S	S	S	R	S
Tigecycline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Nitrofurantoin	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S
Rifampicin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Trimethoprim/Sulfamethoxazole	R	S	S	R	R	R	R	S	R	R	R	R	R	S	S	S	R	S

S = susceptible; R = resistant; I = intermediate susceptibility; - represents negative result; + represents positive result
Isolates were analyzed by VITEK® 2 Compact using the AST-GP71 Card.

APPENDIX D. DISINFECTANTS USED BY EACH FACILITY

A. Disinfectant used at the Long-Term Care Facility

Brand Name: 3M™QUAT DISINFECTANT CLEANER Ready-to-Use (Product No. 5, Twist 'n Fill™ System)

EPA Registration Number: 6836-78-10350

Product: LONZA FORMULATION R-82 (EPA, 2009)

EPA Reg#: 6836-78

Registrant: LONZA INC

Approval Date: 06/19/2007

Active Ingredients:

Dioctyl dimethyl ammonium chloride 2.604%

Alkyl* dimethyl benzyl ammonium chloride*(50%C14, 40%C12, 10%C16) 8.68%

Didecyl dimethyl ammonium chloride 3.906%

Octyl decyl dimethyl ammonium chloride 6.51%

B. Disinfectant used at the Acute Care Hospital

Brand Name: Ecolab's A-456 II Disinfectant Cleaner

EPA Registration: 6836-78-1677

Product: LONZA FORMULATION R-82 (EPA, 2009)

EPA Reg#: 6836-78

Registrant: LONZA INC

Approval Date: 06/19/2007

Active Ingredients:

Dioctyl dimethyl ammonium chloride 2.604%

Alkyl* dimethyl benzyl ammonium chloride*(50%C14, 40%C12, 10%C16) 8.68%

Didecyl dimethyl ammonium chloride 3.906%

Octyl decyl dimethyl ammonium chloride 6.51%

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2. Wright, B. E., Schmidt, K. H., Hunt, A. T., Reschke, D. K., & Minnick, M. F. (2011). Evolution of coordinated mutagenesis and somatic hypermutation in VH5. *Molecular Immunology*, 49(3), 537-548.
3. Wright, B. E., Schmidt, K. H., Davis, N., Hunt, A. T., & Minnick, M. F. (2008). II. Correlations between secondary structure stability and mutation frequency during somatic hypermutation. *Molecular Immunology*, 45(13), 3600-3608.

Abstracts:

1. Tuan~o, K. R., Ezeanolue, E. E., Hunt, A., Shahani, S., Whitten-Shurney, W. (2013). State health department's handling of sickle cell trait results identified through the newborn screening programs. Western Society for Clinical Investigation.
2. Hunt, A., Cruz, P., Buttner, M. P. (2013). The presence of methicillin resistant *Staphylococcus aureus* (MRSA) on environmental surfaces in healthcare facilities pre- and post- cleaning. 6th Annual Interdisciplinary Research Scholarship Day, University of Nevada, Las Vegas.

Thesis Title: The presence of methicillin resistant *Staphylococcus aureus* (MRSA) on environmental surfaces in healthcare facilities pre-and post- cleaning

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