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## **An evaluation of methicillin resistant *Staphylococcus aureus* survival on five environmental surfaces under two different humidities, with and without the addition of bovine serum albumin**

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AN EVALUATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*  
SURVIVAL ON FIVE ENVIRONMENTAL SURFACES UNDER TWO  
DIFFERENT HUMIDITIES, WITH AND WITHOUT THE  
ADDITION OF BOVINE SERUM ALBUMIN

by

Courtney Ann Coughenour

Bachelor of Science  
Pennsylvania State University  
2005

A thesis submitted in partial fulfillment  
of the requirements for the

**Master of Public Health Degree**  
**Department of Occupational and Environmental Health**  
**School of Community Health Sciences**  
**Division of Health Sciences**

**Graduate College**  
**University of Nevada, Las Vegas**  
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**Thesis Approval**  
The Graduate College  
University of Nevada, Las Vegas

March 26, 2009

The Thesis prepared by

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**Entitled**

An evaluation of methicillin resistant Staphylococcus aureus survival  
on five environmental surfaces under two different humidities,  
with and without the addition of bovine serum albumin

is approved in partial fulfillment of the requirements for the degree of

Master of Public Health

*Examination Committee Chair*

*Dean of the Graduate College*

*Examination Committee Member*

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## ABSTRACT

### **An evaluation of methicillin resistant *Staphylococcus aureus* on five environmental surfaces under two different humidities, with and without the addition of bovine serum albumin**

by

Courtney Ann Coughenour

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The spread of antibiotic resistant bacteria is a major public health concern, as they result in greater healthcare costs and increased morbidity and mortality rates. Methicillin resistant *Staphylococcus aureus* (MRSA) is one organism of particular concern, with the number of infections increasing in epidemic proportion. Bacterial surface contamination with MRSA is significant, as it may serve as a reservoir for transmission and have negative health implications. The purpose of this study was to evaluate survival of MRSA on five environmental surface materials; glass, wood, vinyl, plastic, and cloth. The effect of relative humidity (RH) and bovine serum albumin (BSA) were also examined. Surfaces of 5.1cm<sup>2</sup> were inoculated with 3.0 x 10<sup>8</sup>– 1.4 x 10<sup>9</sup> MRSA CFU/ml with and without 1% BSA. Surfaces were incubated at 35°C at 45-55% and 16% RH. Hard surfaces were swab sampled and cloth surfaces hand stomached and re-suspended in phosphate buffer (PB). Suspensions of 100µl were spread plated onto agar plates and incubated at 35°C for 24 hrs; resulting colonies were enumerated. Samples were taken

immediately upon drying (time 0), 3 hrs, 24 hrs, 2 days, 3 days, 4 days, and 5 days. Results showed that there was a significant difference ( $p < .001$ ) among the surfaces; MRSA survived on plastic and vinyl for the longest amount of time, and wood for the least amount of time. The overall greatest concentration of CFU/ml remained on cloth; however, the MRSA did not persist past 3 days. The addition of BSA enabled MRSA to survive significantly longer ( $p < .001$ ). The amount of CFU/ml was significantly less ( $p = .002$ ) on surfaces stored in 45-55% RH versus 16% RH. Information gathered from this study indicates that viable MRSA bacteria can remain on surfaces for days; likely serving as a reservoir for transmission. The best way to control the MRSA epidemic is to prevent transmission.



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but not least I thank my family, I would not be who I am today without their love and support.

## CHAPTER 1

### INTRODUCTION

Until as recently as the early 1900s infectious diseases were the leading cause of death in the U.S., and in some developing nations they still are (World Health Organization [WHO], 2008). With the advent of penicillin it was thought that infectious diseases would become an issue of the past. Not long after the introduction of antibiotics it was found that bacteria possessed the ability to resist such therapies, rendering humans at a constant arms race with microorganisms.

The epidemic of antibiotic resistant bacteria is a major public health issue. Once thought to be a problem solely of the healthcare industry, the emergence of resistant bacteria throughout the community is cause for concern, as resistant infections result in greater healthcare costs and increased morbidity and mortality rates.

Methicillin resistant *Staphylococcus aureus* (MRSA), a resistant strain of a common human pathogen, has gained a great deal of attention recently. With an estimated 1.3 million infections each year and more deaths than those caused by HIV and AIDS combined, MRSA has been nicknamed the “superbug” (Klevens et al, 2007). Understandably, this has garnered a great deal of public fear and anxiety about MRSA and public health interventions are needed to both reduce incidence rates and ease public fear.

Bacterial surface contamination is a matter of public health, as it can cause cases of human illness and disease. Research has found that bacteria are able to persist on environmental surfaces for several months (Kramer et al., 2006). Previous data also indicate that when bacteria are offered organic protection from drying, such as blood or pus, they are able to persist for significantly longer amounts of time (Tolba et al., 2007). It has also been found that as relative humidity (RH) increases, the amount of bacterial surface contamination decreases (Makison & Swan, 2005; Wilkoff et al., 1969). Interestingly, no data exist on the extremely low RH that is common in the Las Vegas Valley and its effect on bacterial surface survivability. In addition, data on MRSA surface survivability are limited.

The goal of this study was to add to the literature on MRSA surface survival focusing on five environmental surfaces; glass, wood, plastic, vinyl, and cloth. In addition, the effect of the extremely low levels of RH common to Las Vegas was compared to other RH values. Furthermore, survival rates were compared with the presence of bovine serum albumin (BSA), a proteinaceous serum used to mimic human proteins which may offer some organic protection.

## CHAPTER 2

### LITERATURE REVIEW

#### Antibiotic Resistant Bacteria

The discovery of penicillin in 1928 began a new era in medicine. Infectious diseases, once the leading cause of death, could now be easily cured by the use of antibiotics. It wasn't long, however, before the first cases of resistant bacteria surfaced (Tan et al., 2000). The ability to resist such drugs began to limit the amount of effective treatments against microorganisms. Antibiotic resistance is not the only problem; there is now a growing concern for organisms which are multi-drug resistant, or resistant to more than one therapeutic drug (Tomasz, 1994). In 2004 it was estimated that 70% of pathogenic bacteria were resistant to at least one antibiotic (Demain & Sanchez, 2009).

Bacterial resistance to antibiotics has ensued from a number of factors. Spontaneous mutations occur quite frequently in microorganisms. Sometimes these mutations can be beneficial to the organism and allow it to survive under otherwise intolerable conditions (National Institute of General Medical Science [NIGMS], 2006). For example, a mutation may arise that alters the shape of the bacterial protein. This shape change may affect the antibiotics ability to bind to the target protein, thus the drug will no longer be successful at inactivating the protein. Mutations may take place which allow the bacteria to produce an antibiotic inactivating enzyme or change the permeability of the bacterial cell wall, which, in turn, renders such antibiotics ineffective against that microorganism.



Resistance may also be attained via a gene transfer from one bacterium to another. This transfer can take place both among and between bacterial species (NIGMS, 2006).

Mutations and gene transfers have taken place for millions of years; however, the recent exponential growth in the number of resistant bacteria is the result of unnecessary use of such drugs, be it from human or veterinary medicine (Trepka et al., 2000). Antibiotic use selects for resistance by eliminating strains susceptible to that drug. If there happens to be any resistant bacteria present, they are now free to multiply without any competition from the susceptible strains (NIGMS, 2006).

The spread of antibiotic resistant bacteria has recurrently been a concern in clinical settings, but more recently has become a concern in community settings as well. The spread of resistant bacteria throughout the community is an essential public health issue, as such infections are often more difficult to treat and result in higher morbidity and mortality rates (Romero-Vivas et al., 1995). In addition, the length of hospital stay and the costs associated with the treatment of antibiotic resistant bacterial infections are nearly double that of susceptible infections (Lodise & McKinnon, 2005).

#### Methicillin Resistant *Staphylococcus aureus* [MRSA]

A resistant strain of *Staphylococcus aureus*, a common human pathogen, has recently gained increased attention. Garnering the nickname the “superbug”, this organism is unquestionably a leading public health concern.

*S. aureus* is a spherical, golden-colored bacterium of the *Bacillaceae* family. It often comprises part of the normal human flora, meaning that the bacteria are present on the human body, but cause no harmful health effects. Most commonly it is found in the nose

and on the skin of healthy individuals (Centers for Disease Control and Prevention [CDC], 2005). Approximately 32% of the U.S. population is colonized in the anterior nares (Kuehnert et al., 2005). *S. aureus* is an opportunistic pathogen; it normally does not cause disease in healthy individuals. However, the immunocompromised may be at risk for infection (Kloos & Bannerman, 1994).

The first resistant strains of *S. aureus* were reported in 1961; they were resistant to methicillin and other commonly used antibiotics in the penicillin family, subsequently named methicillin resistant *Staphylococcus aureus*, or MRSA (Sutherland & Rolinson, 1964). Resistant strains possess the *mecA* gene, contained in the staphylococcal cassette chromosome *mec* (SCC*mec*) (Emmanuel, 2003). This gene enables the bacteria to overcome the essential beta-lactum ring found in penicillins by one of two means. Resistant bacteria can either produce a beta-lactamase enzyme, giving it the ability to break the beta-lactum ring; or they can produce a modified cell-wall synthesizing enzyme, allowing the bacteria to synthesize cell-walls and reproduce uninhibited by the presence of the drug (Emmanuel, 2003).

MRSA has become endemic in clinical settings, and has recently emerged in the community setting. When first discovered MRSA was confined to hospital acquired infections [HA-MRSA]; meaning that infected individuals had been hospitalized or involved in a healthcare facility within a year of the infection (Klevens et al., 2007). These are usually individuals that already have weakened immune systems such as the elderly or chronically ill patients. Recently, more infections of young, healthy individuals having no interaction with the healthcare setting have been emerging. These

infections are referred to as community acquired MRSA [CA-MRSA] (Klevens et al., 2007).

The most common mode of transmission is person to person contact, most often via the hands. The spread of the bacteria occurs when an individual comes in contact with an infected lesion or nasal discharge from a colonized individual. The bacteria can be easily transferred if proper hand sanitation does not take place. Transmission may also occur from surface contamination such as contaminated objects or fomites (CDC, 2007).

Numerous experimental data have shown that MRSA strains may behave differently from antibiotic susceptible *S. aureus* strains (Klevens et al., 2006; Siegel et al., 2006; Sutherland & Rolinson, 1964). Individuals colonized with MRSA are four times more likely to develop symptomatic infections when compared to those colonized with susceptible *S. aureus* (Furlow, 2009). MRSA infections are also associated with higher case mortality rates. This may be caused by a delay in treatment with effective antibiotics such as vancomycin or a decreased bactericidal activity of such antibiotics (Siegel et al., 2006). Consequently, surface contamination with MRSA is of significant public health concern.

### Bacterial Surface Contamination

Bacterial surface contamination is a public health concern as it has been implicated to cause cases of human disease (Griffith et al., 2003; Rutala & Weber, 2001; Davies et al., 2000; Oie & Kamiya, 1996). Data show that bacteria are able to persist in the environment, surviving on surfaces and fomites for six months or longer (Kramer et al., 2006). Such surface contamination may occur from the expulsion of microbial cells

during sneezing, coughing and talking; about 3,500 infectious droplets are expelled during a cough and up to one million during a single sneeze (Ait-Khaled & Enarson, 2003). The moisture in the droplet evaporates leaving behind the bacterial cells which can be transmitted via fomites and inanimate objects. The resulting surface contamination poses a risk for transmission of human illness (Rutala & Weber, 2001; Rusin, 2002).

The literature on the survivability of MRSA on surfaces is limited, though previous data have found that it can persist on dry surfaces for up to seven months (Kramer et al., 2006). Neely & Maley (2000) found that MRSA can persist from days to months on fabrics. Oie et al. (2005) found that disinfection was effective for MRSA contamination on smooth surfaces, but that significant amounts of bacteria remained on porous surfaces. Overall, the properties of the surface type appear to have an influence on survival rates.

There are conflicting data on virulence factors of MRSA and their ability to aid in survival. Some studies have found no significant differences in survival rates (Duckworth & Jordens, 1990), while other studies describe MRSA to be more resistant to dehydration than sensitive *S. aureus* strains (Rozgonyi et al., 2007; Makison & Swan, 2005).

Tolba et al. (2007) found that after inoculation of MRSA onto coins and glass surfaces, no viable organisms could be detected after four hours. Suspecting that the bacteria could not withstand the drying, they inoculated the surfaces again with the addition of blood or pus for organic protection. It was found that survival was significantly enhanced by an additional 13 days, after which the study was terminated.

This finding is significant as most often bacteria are deposited onto surfaces with some organic material that may provide some protection.

Research has indicated that relative humidity (RH) can have an effect on the survival rates of bacteria (Makison & Swan, 2005; Wilkoff et al., 1969). It has been shown that the survival rate of *S. aureus* decreases to below 40% at 95% RH, yet remains over 70% at 35% RH (Makison & Swan, 2005). Wilkoff et al. (1969) also showed that a RH of 35% allowed organisms to remain viable for significantly longer than a RH of 78%. Overall, it has been found that the death rate of most bacteria increases as the RH increases. Interestingly, no studies are published examining the impact of survival on RH below 35%, an occurrence which is common in the Las Vegas Valley.

Knowledge on the length of time MRSA bacterial surface contamination remains and the factors that affect it will serve useful in developing prevention measures. The longer the bacterial surface contamination remains, the longer it can serve as a source for transmission of disease and ill health effects.

#### The Public Health Concern of MRSA

There are a number of factors that deduce an issue as a public health concern. If the issue affects the health and well being of a large number of individuals and the incidence rates are significantly above the norm, the issue is a matter of public health (Chino, 2007). If the issue will affect a larger number if it is not addressed, if treatment is difficult, yet prevention is effective, or if it engenders a great deal of public fear or concern, it is an issue for public health. If the problem illustrates large economic consequences or is associated with large expenditures of public or private funds to

combat it, it is a concern for public health. Furthermore, it is a public health concern if specific high risk populations are disproportionately affected when compared to the rest of the population (Chino, 2007). MRSA fits into all of the above and is discussed in detail below.

The numbers of MRSA infections are increasing in epidemic proportion. In 1974 MRSA accounted for 2% of all *S. aureus* infections, increasing to 22% in 1995, and in 2004 the number of MRSA infections reached 64% (Klevens et al., 2006). Moran et al. (2006) reported that MRSA accounted for 59% of all skin and soft tissue infections in 11 U.S. cities. Extrapolation of data from Klevens et al. (2007) suggests that there may be over 1.3 million MRSA infections each year. It is important to note that MRSA is not listed as a reportable condition so not all states report MRSA to the Centers for Disease Control and Prevention (CDC). Therefore, the above data only represent those numbers from institutions which voluntarily reported infection rates to the CDC.

With the unmistakable epidemic of HA-MRSA and the increasing amount of CA-MRSA, it is unlikely that infection rates will drop without intervention. Prevention is the key to a decrease in the number of infections; a disruption in the transmission of the bacteria will dramatically reduce the amount of new infections. Public health efforts should be established to aid in prevention.

In 2005 MRSA took the life of over 18,000 individuals; this is more than the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) combined (Furlow, 2009). With such astounding figures, the media has set in motion a whirlwind of press surrounding MRSA, dubbing it the “superbug”. Understandably, a

great deal of public fear and anxiety about the issue has ensued. Such public fear renders public health efforts to combat the problem critical.

If the problem results in large economic consequences it is a concern for public health. The average length of hospitalization for individuals with MRSA infections is more than double that for non-MRSA stays (Elixhauser & Steiner, 2007). In addition, MRSA hospitalizations cost nearly double that of non-MRSA stays; \$14,000 for MRSA stays versus \$7,600 for non-MRSA stays (Elixhauser & Steiner, 2007). A recent policy adopted by Medicare and Medicaid has eliminated hospital reimbursement for treatment of hospital acquired infections (Evans, 2008). In an attempt to sustain payments, some hospitals have adopted pre-admission screening for MRSA colonization in hopes to confirm that not all infections are HA-MRSA (Evans, 2008). Such increased costs for insurance companies and hospitals will have to be recouped somewhere, and will ultimately end up in higher insurance premiums and out of pocket expenses for the overall public.

#### Vulnerable Populations and MRSA

There are a number of populations that are specifically vulnerable to MRSA infections, though the populations vary depending on whether the infection is CA-MRSA or HA-MRSA. This comes as no surprise given the differing risk factors for each infection. The major risk factors for CA-MRSA infections include skin to skin contact, cuts or abrasions in the skin, crowded living conditions, poor hygiene and contaminated surface items (CDC, 2005). Risk factors for HA-MRSA include involvement in a healthcare or long term care facility, invasive devices such as catheters or feeding tubes, and recent antibiotic use (Mayo Clinic, 2008).

Children are among the vulnerable populations at an increased risk of developing MRSA infections, and are of particular concern because they can quickly develop into a serious, widespread infection (CDC, 2005). Risk factors such as cuts or abrasions in the skin, close skin to skin contact, and poor hygiene impact infection rates in children. In childcare facilities, contact by children and personnel with contaminated surfaces may also serve as a reservoir for MRSA, posing serious risk of transmission and infection (Rutala and Weber, 2001). The presence of bacteria on children's toys is of significance, as it has been implicated to cause outbreaks of infection. Davies et al. (2000) demonstrated that 98% of toys in one neonatal intensive care unit were colonized with bacteria, Buttery et al. (1998) reported an outbreak in a children's oncology ward of a drug resistant bacteria from bath toys, and Avila-Aguero et al. (2004) showed that toys entering the hospital were already colonized with at least one pathogenic bacterium. Data on surface contamination can be applied to childcare facilities; not only in the sense of toys, but also items such as sleeping cots, changing tables, blankets, and flooring.

Those involved in long term care facilities are at an increased risk for developing infections. Such facilities have elevated rates of resistant bacteria due to the high use of antibiotics and possible genetic mutations within the facility (Strausbaugh et al., 1996). MRSA is of great concern, as it is often endemic and can result in outbreaks (Muder et al., 1991). All of the surfaces being tested in this study can be applied to surfaces and objects located in long-term-care facilities and can aid in prevention planning for the minimization of outbreak occurrences.

The elderly, along with immunocompromised individuals are vulnerable to MRSA infections. This population is more likely to have involvement with healthcare facilities



and prior use of antibiotics, both risk factors for infection. Mortality from invasive infection in the elderly is twice that of younger patients with invasive MRSA. One potential confounder is that the elderly are less likely to present with a fever when afflicted with an invasive MRSA infection, this can result in a delay of effective treatment. Moreover, the elderly are likely to have underlying disease that can add to poor overall health (Tacconelli & Cataldo, 2007).

Numerous MRSA outbreaks in hospitals have been documented. Surface contamination has been implicated as a source of infection from floors, linens, medical equipment, furnishings, and inanimate objects (Shiomori et al., 2001). Healthcare worker gowns and patient care items have also been implicated as sources of contamination. Boyce et al. (1997) found that 73% of hospital rooms with patients infected with MRSA and 69% of rooms with colonized patients contained surface contamination. Additionally, it was found that nurse's gloves became contaminated 42% of the time after contact with contaminated surfaces. This is of importance, as many individuals in hospitals are already in an immunocompromised state. Also, surgical wound infections or infections in burn units may develop rapidly to invasive disease and often result in high morbidity and mortality. Knowing surface survivability will help to contain and minimize hospital infections and outbreaks.

Overweight individuals are more vulnerable when it comes to developing MRSA infections. The exact reason is unknown, but it is hypothesized that being overweight results in different patterns of skin colonization which increases the likelihood of infection (Turabelidze et al., 2006).

Athletes are another vulnerable population when it comes to CA-MRSA infections. This is contributable to the high rates of skin to skin contact involved in most sports (Benjamin et al., 2007). Sporting facilities such as locker rooms, showers, weight rooms and equipment can serve as potential reservoirs for MRSA. Information on surface contamination will be applicable to sporting facilities to decrease the risk of MRSA transmission.

Prisoners and military personnel are vulnerable to MRSA infections due to the crowded conditions of most communal living facilities. One study found that isolates from a prison were virtually indistinguishable, suggesting that transmission of one particular strain within the facility was the cause of the outbreak (Aiello et al., 2006). In a study of military recruits, having a roommate with an MRSA infection was a significant risk factor, suggesting that person to person transmission or surface contamination may influence infection rates (Aiello et al., 2006).

There are racial disparities in the amount of MRSA infections. Klevens et al. (2007) found that blacks had a significantly higher rate of MRSA infection as compared to whites among all age groups. However, little is known as to why such racial disparities exist.

Without attention to these health disparities, vulnerable populations will continue to experience higher rates of MRSA infections. Consequently, the U.S. will continue to spend more yet have far poorer health than many other developed nations (Shi & Stevens, 2005).

## Controlling the Epidemic

The best way to control the epidemic and decrease the incidence rate of MRSA infections is to prevent them. The key to prevention is breaking the mode of transmission. There are a number of effective ways to do this. A primary level of prevention includes educating the public on what they can do to minimize their own risks of exposure. Strategies to minimize risk include (CDC, 2005):

- Keeping hands clean by thorough washing or using an alcohol based sanitizer.
- Keeping abrasions clean and covered until healed.
- Avoiding contact with others wounds or bandages.
- Not sharing personal items such as towels or razors.
- Showering after athletic work outs.
- Using a barrier such as a towel between skin and shared equipment.
- Cleaning the surface of equipment before and after use.
- Asking doctors and nurses to wash hands before interacting with them in healthcare situations.

A secondary prevention method would consist of early detection as to what antibiotics the organism is susceptible to by taking an initial culture of all forms of the infection (Lesseva & Hadjiiski, 1996). With early detection the proper treatment could be provided in a timely manner and a decrease in the overall mortality from infections could be achieved. Another secondary prevention method is routine eradication of bacteria from healthcare workers and known MRSA colonized and infected patients (Lessing et al., 1996). Eradication is achieved via the use of specific antiseptic soaps and nasal sprays (Boyce, 2001).

Novel research has recently emerged which shows that exposure to visible light, not of the dangerous UV spectrum, has the ability to inactivate MRSA (Maclean et al., 2008; Enwemeka et al., 2008). This may have potential implications on new effective ways to treat skin infections, colonization, and disinfect surfaces. Further research is still needed.

Antibiotic resistant bacteria such as MRSA are a major public health concern. Primary prevention methods in which individuals can avoid infection from the bacteria initially seem to be the best option at decreasing incidence rates. Education programs should be implemented aimed at educating the public of prevention measures within their control. These programs should be universal; however, at risk populations should be heavily targeted. The amount of MRSA infections are increasing at epidemic proportions and public health interventions are needed to bring to an end to it.

## CHAPTER 3

### OBJECTIVES, QUESTIONS, AND HYPOTHESES

#### Objectives

The objective of this study was to determine the variability of survival of MRSA on five environmental surface materials; glass, wood, vinyl, plastic, and cloth. Survival of bacterial cells on surfaces stored in an artificially amplified humid environment was compared to those stored in the naturally arid Las Vegas Valley relative humidity to determine if humidity had an effect on survival time. In addition, a comparison was done on survivability time on surfaces inoculated with MRSA culture/buffer only solution versus surfaces inoculated with MRSA culture/buffer solution amended with 1.0% bovine serum albumin (BSA), a proteinaceous serum. The addition of BSA serves to mimic human protein, as if the bacteria were protected by a human secretion to determine if the presence of proteins has an effect on survival time. The overall objective is to add to the body of literature on bacterial surface contamination to aid in public health efforts to minimize transmission of MRSA and decrease human illness.

#### Questions

Will MRSA survivability differ between the five different surface types?

Does relative humidity have an effect on survival time?

Will the addition of BSA increase the amount of time that MRSA survives on the surfaces?

Will there be an interaction effect between relative humidity and the presence of a protein such as BSA?

### Hypotheses

Hypothesis 1: The survival time of MRSA will vary on the different environmental surfaces.

$H_0$ : The survival time of MRSA is not significantly different amongst the different surface types.

$H_a$ : The survival time of MRSA differs significantly amongst the different surface types.

Hypothesis 2: The survival time of MRSA will be longer on the cloth (porous) surfaces when compared to the hard surfaces.

$H_0$ : The survival time of MRSA is not significantly higher on the cloth surfaces when compared to the hard surfaces.

$H_a$ : The survival time of MRSA is significantly higher on the cloth surfaces when compared to the hard surfaces.

Hypothesis 3: The addition of 1.0% BSA to the MRSA culture solution will have a positive impact on survival time.

$H_0$ : The survival time of MRSA is not significantly different in the culture solution amended with 1.0% BSA vs the culture only solution.

$H_a$ : The survival time of MRSA is significantly higher in the culture solution amended with 1.0% BSA vs. the culture only solution.

Hypothesis 4: MRSA will survive longer on surfaces when stored in the artificially enhanced humid environment.

$H_0$ : The survival time of MRSA at 16% RH is not significantly different from the survival time at 45-55% RH.

$H_a$ : The survival time of MRSA is significantly higher in the artificially enhanced humid environment

## CHAPTER 4

### MATERIALS AND METHODS

#### Experimental Design

To evaluate the survival ability of MRSA on five environmental surfaces under differing conditions, survival rates were compared in a series of laboratory experiments. Survival rates of MRSA on surfaces stored in an artificially amplified humid environment were compared to those stored in the naturally arid Las Vegas Valley relative humidity. Additionally, a 1.0% solution of filter-sterilized bovine serum albumin (BSA) was added to the bacterial culture before application onto the surfaces. Survival was compared with and without the addition of the serum.

#### Test Organisms and Culture Media

The test organism was obtained from American Type Culture Collection (ATCC; Manassas, VA); *Staphylococcus aureus* ATCC 43300 (MRSA). This particular strain was chosen as it is commonly used in the current experimental data on MRSA (Felten et al., 2002; Louie et al., 2000; Reischl et al., 2000). The strain was a clinical isolate, first identified in Kansas. It is heterogeneous in its oxacillin resistance, meaning that a minority of cells express the resistance phenotype, but the entire population carries the genetic markers for it. There are conditions that may enhance or minimize the expression



of resistance, though these changes are transient and reversible due to the organism's ability to induce beta-lactum resistance mechanisms.

The organism was cultured on Tryptic Soy Agar (TSA, Difco™, Sparks, MD) and Tryptic Soy Broth (TSB, Bacto™, Sparks, MD) and incubated at 35°C for 24 to 96 hours. *S. aureus* is a human pathogen, thus blood agar is often the media of choice. However, in a small comparison study of the two different mediums and bacterial surface recovery, there was no significant difference (data not shown). TSA was consequently chosen due to lower cost and longer shelf life. The culture was suspended, washed and re-suspended in a sterile 0.01M phosphate buffer (PB, Fisher Scientific, Fair Lawn, NJ). In comparative experiments, the final washed culture was re-suspended in PB containing 1.0% BSA (Rockland, Philadelphia, PA). The BSA was used in an attempt to mimic human proteins, such as saliva, pus, or mucus. Such proteins can potentially offer the bacteria protection during surface contamination and lengthen the time of survival.

#### Test materials

MRSA survivability was tested on common environmental surfaces including: non-PVC plastic cutting boards, glass petri-plates, wood cutting boards, vinyl flooring tiles, and flannel cloth purchased at a local fabric store. Vinyl, plastic, and wood surfaces were divided into 5.1cm squares, separated by ¼ inch wide laboratory tape. Glass petri-plate squares were distinguished by a marker line drawn on the dish opposite the sampling surface. Cloth surfaces were cut into 5.1cm squares and sewn together with a low loft 100% cotton quilting batting sandwiched in between the two flannel layers. For each surface the squares were tested under varying conditions: four squares maintained at 45-

55% RH with BSA, four squares maintained at 45-55% RH without BSA, four squares maintained at 16% RH with BSA, and four squares maintained at 16% RH without BSA. This set of conditions was repeated at seven different time intervals. This resulted in 112 squares being tested for each surface and 560 squares tested for all surfaces combined.

### Humidity Maintenance

A pan of tap water was placed into the incubator to maintain a RH of 45 to 54%, which was monitored with a humidity probe (model #22046982, Fisher Scientific, Fair Lawn, NJ).

### Procedure

For each experiment a culture was grown by streaking freezer stock of MRSA strain ATCC 43300 onto TSA and incubating at 35°C for 24 to 96 hours. An overnight culture was prepared by inoculating 20ml of TSB with approximately five bacterial colonies and incubating at 35°C at 60rpm for approximately 15 hours in an orbital incubator shaker (Amerex Instruments, Lafayette, CA). A working culture was prepared by inoculating 100ml of TSB with the overnight culture and incubating at 35°C at 200rpm. The optical density (OD) was measured using a spectrophotometer (Beckman Coulter, Fullerton, CA). The spectrophotometer determines the optical density by measuring the amount of radiant energy passing through the suspension as compared to a blank sample. The OD was read at periodic intervals until an absorbance OD of 0.9 to 1.0 was obtained. At this OD cells were in the late log phase, thus allowing the highest concentration of viable cells to be obtained.

Twenty-five ml of culture was then harvested and centrifuged at 10,000 x g for five minutes at room temperature. The supernatant was removed and the bacterial cell pellet washed and re-suspended in 25ml of PB two additional times. The final washed culture was either re-suspended in PB or PB containing 1.0% BSA. It was then enumerated via serial dilutions and spread plating 100µl to ensure a cell concentration of  $3.0 \times 10^8$ –  $1.4 \times 10^9$  colony forming units (CFU)/ml. Spread plating is performed by inoculating 100µl of liquid into the middle of the agar plate. A glass L-shaped rod is then moved continually over the surface of the agar while the plate spins. This allows the amount of liquid to be distributed evenly over the surface of the agar, thus enabling isolated colonies to grow and the number of CFU can be enumerated.

A 100µl aliquot of the culture was pipetted onto the 5.1cm<sup>2</sup> of an environmental surface, spread with a disposable sterile plastic loop and allowed to dry at room temperature. Sampling took place immediately upon drying (time 0), 3hrs, 24hrs, 2days, 3days, 4days, and 5days. Two methods of sampling were performed on hard surface areas (plastic, glass, wood, and vinyl). Immediately upon drying two TSA-filled replicate organism detection and counting (RODAC) contact samples were applied with even pressure to each 5.1cm<sup>2</sup> of surface, a lid was placed over the RODAC and the plates were incubated at 35°C for 24 hours. Subsequently, two sterile cotton tipped swabs were moistened in 500µl of PB and used to swab additional 5.1cm<sup>2</sup> of surface in two directions at right angles to each other. Each swab was then individually processed by placing in 1ml of PB and vortexing (VWR VM-3000 Mini Vortex, West Chester, PA) for one minute to release MRSA cells into suspension.

For cloth, each surface was individually processed in a sterile stomacher bag with 10ml of PB and hand stomached for one minute to release MRSA cells into suspension. For all surfaces, serial dilutions were made in PB and 100µl spread plated onto TSA. Plates were then placed in a 35°C incubator for 24 hours. Surfaces were stored in an incubator which was maintained either at 45 to 55% RH or a non-humidified incubator that was similar to the Las Vegas arid climate; throughout the experiment the humidity in the non-modified incubator remained at 16%. The resulting colonies were then enumerated to determine the number of CFU/ml. Replicate experiments were performed for each surface.

#### Quality control

To ensure that there was no prior contamination of the surfaces, a quality control (QC) was performed with each experiment. For each hard surface one square was left blank, thus received no aliquot of the culture. A swab sample was taken of such square and processed in the same manner as previously mentioned. For cloth surfaces, one fabric square received no culture and was processed as previously mentioned. A QC was also performed on the buffers being used (PB and PB amended with 1.0% BSA) to ensure that there was no contamination. If the QC's consisted of no growth after 24 hours, it was presumed that the materials were free from contamination.

#### De-contamination

All hard surfaces were decontaminated by soaking in a 20% bleach solution for approximately 15 minutes. They were then rinsed and soaked in clean water for

approximately 15 minutes to ensure no bleach residue was left on the surface. Upon drying, surfaces were wiped with 70% isopropyl alcohol under a laminar flow hood, and then further sterilized under an ultraviolet germicidal light for 30 minutes. Cloth surfaces were autoclaved and discarded after sampling.

### Statistical Analysis

SPSS for Windows® Version 15.0 was used to perform statistical tests on the data. A repeated measures analysis of variance (ANOVA) was chosen because the same organism was measured under a number of different conditions over time. The number of CFU/ml was measured at several different time intervals; therefore, time is the repeated factor. The between subjects factor is the surface type; glass, wood, vinyl, plastic, and cloth. Tukey's post hoc test was performed for comparison of each surface. The within subjects factors are humidity type and culture type (either with or without BSA). For a repeated measures ANOVA, sphericity is required, accordingly Mauchly's Test of Sphericity was performed. Sphericity states that the correlations between each time interval should be similar. For example, the decrease in the amount of CFU/ml should be similar from time 24 hours to the decrease in CFU/ml at time 2 days. However, sphericity is often violated since things measured closer in time tend to be more highly correlated than those measured farther away in time. In this instance a Greenhouse-Geisser Epsilon correction factor was applied. This correction factor adjusts the degrees of freedom in the ANOVA to obtain a more accurate significance value for a close estimation of sphericity (Tabachnick & Fidell, 2007).

## CHAPTER 5

### RESULTS

Prior to data analysis, data were  $\log_{10}$  transformed to ensure normality. The duplicate measurements of CFU/ml over time for the BSA/no BSA and two RH values for each of the surfaces were averaged (Table 1). From a starting cell concentration of  $3.0 \times 10^8$ –  $1.4 \times 10^9$  CFU/ml of MRSA, there was generally a steep decline in the overall mean on all surfaces (Figure 1). The study was terminated at 5 days because there was minimal growth persisting at this time as measured by MRSA CFU/ml. There was a significant difference among the different surface types ( $p < .001$ ). MRSA survived the longest on plastic and vinyl, persisting up to five days. At five days there was a total mean of 2.5 CFU/ml on plastic and 9.4 CFU/ml on vinyl. The rate of decline was greatest on wood with no CFU/ml present at 2 days, followed by cloth which had no CFU/ml present at 4 days. The detection limit (DL) was 1 CFU/ml. Tukey's post hoc test showed that the overall mean was significantly lowest for wood ( $p < .001$ ) as compared to all other surfaces and highest for cloth ( $p < .001$ ) with a mean difference of  $9.7 \times 10^5$  CFU/ml ( $\pm 7.1 \times 10^4$  S.E.).

The addition of BSA had a significant positive effect on time of survival as compared to culture with no BSA ( $p < .001$ ) with an overall mean difference of  $2.8 \times 10^5$  CFU/ml ( $\pm 3.3 \times 10^4$  S.E.). The mean of CFU/ml were initially higher at time 0 and remained so throughout day 5 (Figure 2).

There was a significant interaction effect with surface type and culture type. The concentration of MRSA CFU/ml was significantly higher ( $p < .001$ ) with the addition of BSA on all surface types (Figure 3). The means of MRSA CFU/ml with and without BSA are listed in Table 2.

Increased humidity (45-55%) had a significant negative effect on survival time ( $p = .002$ ) as compared to ambient 16% RH, with an overall mean difference of  $1.5 \times 10^4$  CFU/ml ( $\pm 8.7 \times 10^3$  S.E.) (Figure 4).

There was also a significant interaction effect ( $p < .001$ ) with surface type and RH overall. Individually, plastic and vinyl surfaces were significantly different. For these surfaces the concentration of CFU/ml was significantly lower with the increased RH of 45-55% (Figure 5). The means of MRSA CFU/ml in regards to humidity on plastic and vinyl are listed in Table 3.

There was no significant interaction effect with humidity and BSA/no BSA; no specific combinations of RH and BSA/no BSA resulted in a change in survivability of MRSA as measured by CFU/ml.

The results of the RODAC contact sampling mimicked the results of the swab sampling method (Table 4). However, no statistical analysis was performed on this data, as results were used as a qualitative method rather than a quantitative method.

Table 1: Average concentration of methicillin-resistant *Staphylococcus aureus* (CFU/ml) over time on five surface types at two relative humidities (RH) with and without bovine serum albumin (BSA) (DL=1 CFU/ml).

Surface type	RH (%)	BSA	CFU/ml $\pm$ 1 S.E.						
			Time 0	3 hrs	24 hrs	2 day	3 day	4 day	5 day
Glass n=16	45-55%	-	$1.1 \times 10^6 \pm 5.6 \times 10^5$	$3.9 \times 10^3 \pm 4.4 \times 10^3$	20.0 $\pm$ 33.7	2.5 $\pm$ 5.0	<DL	<DL	<DL
		+	$2.3 \times 10^5 \pm 1.4 \times 10^5$	$1.9 \times 10^4 \pm 1.6 \times 10^4$	$1.7 \times 10^3 \pm 1.5 \times 10^3$	$1.1 \times 10^2 \pm 81.8$	<DL	<DL	<DL
	16%	-	$8.3 \times 10^5 \pm 4.0 \times 10^5$	$2.1 \times 10^3 \pm 1.3 \times 10^3$	37.5 $\pm$ 62.4	<DL	<DL	<DL	<DL
		+	$2.1 \times 10^4 \pm 1.1 \times 10^4$	$4.2 \times 10^3 \pm 3.8 \times 10^3$	$2.8 \times 10^2 \pm 2.4 \times 10^2$	67.5 $\pm$ 45.0	7.5 $\pm$ 5.0	42.5 $\pm$ 49.2	<DL
Wood n=16	45-55%	-	$1.5 \times 10^5 \pm 1.6 \times 10^5$	$9.1 \times 10^2 \pm 1.1 \times 10^3$	5.0 $\pm$ 5.8	<DL	<DL	<DL	<DL
		+	$3.9 \times 10^4 \pm 3.7 \times 10^4$	$3.4 \times 10^2 \pm 1.6 \times 10^2$	10.0 $\pm$ 14.1	<DL	<DL	<DL	<DL
	16%	-	$6.5 \times 10^3 \pm 3.0 \times 10^3$	60.0 $\pm$ 52.9	2.5 $\pm$ 5.0	<DL	<DL	<DL	<DL
		+	$2.9 \times 10^4 \pm 4.3 \times 10^4$	$4.2 \times 10^3 \pm 3.5 \times 10^3$	32.5 $\pm$ 26.3	<DL	<DL	<DL	<DL
Plastic n=16	45-55%	-	$3.7 \times 10^4 \pm 5.3 \times 10^3$	22.5 $\pm$ 26.3	<DL	<DL	<DL	<DL	<DL
		+	$6.4 \times 10^4 \pm 7.0 \times 10^3$	$1.3 \times 10^4 \pm 1.5 \times 10^3$	$1.3 \times 10^3 \pm 3.6 \times 10^2$	45.0 $\pm$ 26.5	2.5 $\pm$ 5.0	<DL	<DL
	16%	-	$3.2 \times 10^4 \pm 1.2 \times 10^4$	$8.9 \times 10^3 \pm 5.9 \times 10^3$	$1.7 \times 10^2 \pm 1.9 \times 10^2$	42.5 $\pm$ 53.2	10.0 $\pm$ 14.1	<DL	<DL
		+	$1.4 \times 10^4 \pm 1.1 \times 10^4$	$4.5 \times 10^3 \pm 3.6 \times 10^3$	$6.4 \times 10^2 \pm 3.5 \times 10^2$	$2.4 \times 10^2 \pm 1.6 \times 10^2$	80.0 $\pm$ 57.2	37.5 $\pm$ 37.8	10.0 $\pm$ 11.5
Vinyl n=16	45-55%	-	$3.7 \times 10^4 \pm 4.3 \times 10^4$	17.5 $\pm$ 17.1	<DL	<DL	<DL	<DL	<DL
		+	$1.5 \times 10^5 \pm 2.2 \times 10^5$	$5.7 \times 10^3 \pm 4.9 \times 10^3$	$5.1 \times 10^2 \pm 3.3 \times 10^2$	$1.8 \times 10^2 \pm 1.0 \times 10^2$	32.5 $\pm$ 12.6	<DL	<DL
	16%	-	$1.2 \times 10^3 \pm 6.8 \times 10^2$	$4.7 \times 10^2 \pm 2.2 \times 10^2$	<DL	<DL	<DL	<DL	<DL
		+	$1.7 \times 10^4 \pm 6.3 \times 10^3$	$5.4 \times 10^3 \pm 1.2 \times 10^3$	$1.6 \times 10^3 \pm 5.8 \times 10^2$	$8.7 \times 10^2 \pm 9.2 \times 10^2$	$2.1 \times 10^2 \pm 1.5 \times 10^2$	75.0 $\pm$ 73.3	37.5 $\pm$ 38.6
Cloth n=16	45-55%	-	$2.2 \times 10^6 \pm 1.7 \times 10^6$	$1.2 \times 10^5 \pm 1.2 \times 10^5$	$3.7 \times 10^3 \pm 2.3 \times 10^3$	$3.5 \times 10^2 \pm 1.7 \times 10^2$	75.0 $\pm$ 96.0	<DL	<DL
		+	$9.9 \times 10^6 \pm 1.8 \times 10^6$	$4.8 \times 10^5 \pm 3.5 \times 10^5$	$1.5 \times 10^4 \pm 1.0 \times 10^4$	$1.5 \times 10^2 \pm 1.7 \times 10^2$	<DL	<DL	<DL
	16%	-	$7.0 \times 10^5 \pm 6.2 \times 10^5$	$2.5 \times 10^3 \pm 1.8 \times 10^3$	$4.3 \times 10^3 \pm 8.4 \times 10^2$	$4.7 \times 10^2 \pm 9.5 \times 10^1$	$3.5 \times 10^2 \pm 3.0 \times 10^2$	<DL	<DL
		+	$1.4 \times 10^7 \pm 5.8 \times 10^6$	$2.1 \times 10^5 \pm 1.5 \times 10^5$	$1.9 \times 10^3 \pm 2.2 \times 10^3$	$2.3 \times 10^2 \pm 1.5 \times 10^2$	50 $\pm$ 58.0	<DL	<DL



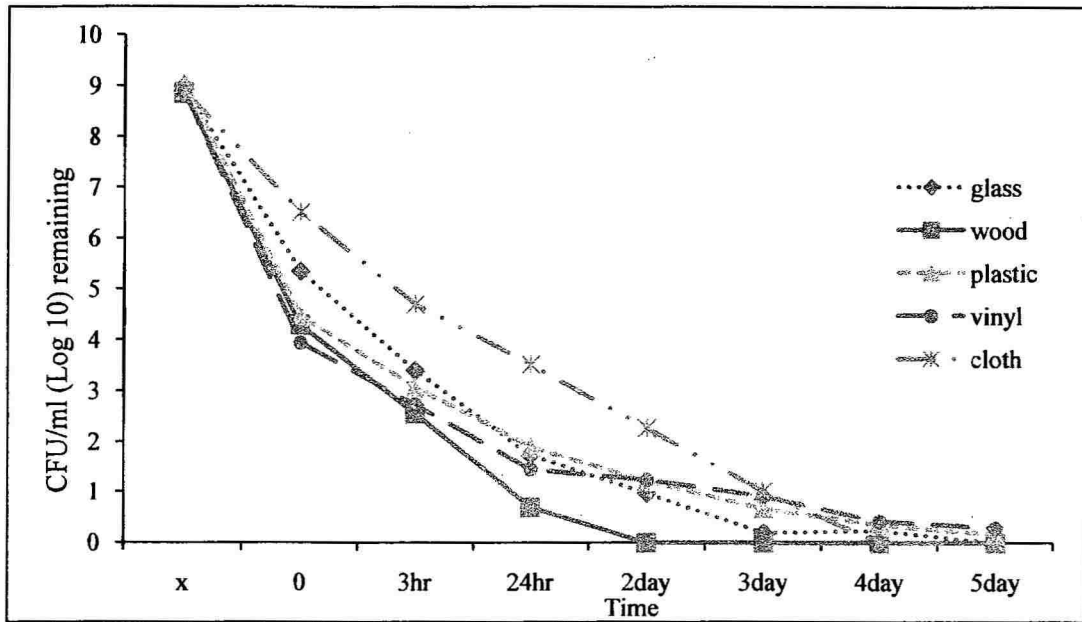


Figure 1: Overall average concentration of methicillin-resistant *Staphylococcus aureus* ( $\text{Log}_{10}$  CFU/ml) over time on five surface types (x = concentration at inoculation; time 0 = at drying).

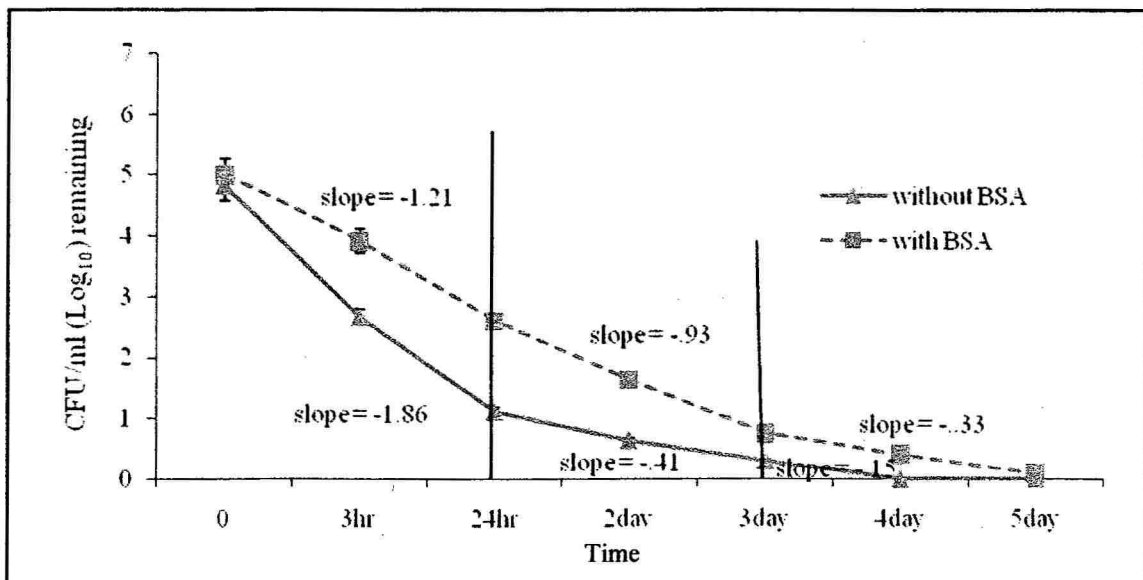


Figure 2: Average concentration of methicillin-resistant *Staphylococcus aureus* ( $\text{Log}_{10}$  CFU/ml) remaining over time with and without bovine serum albumin (BSA) for all five surface types.

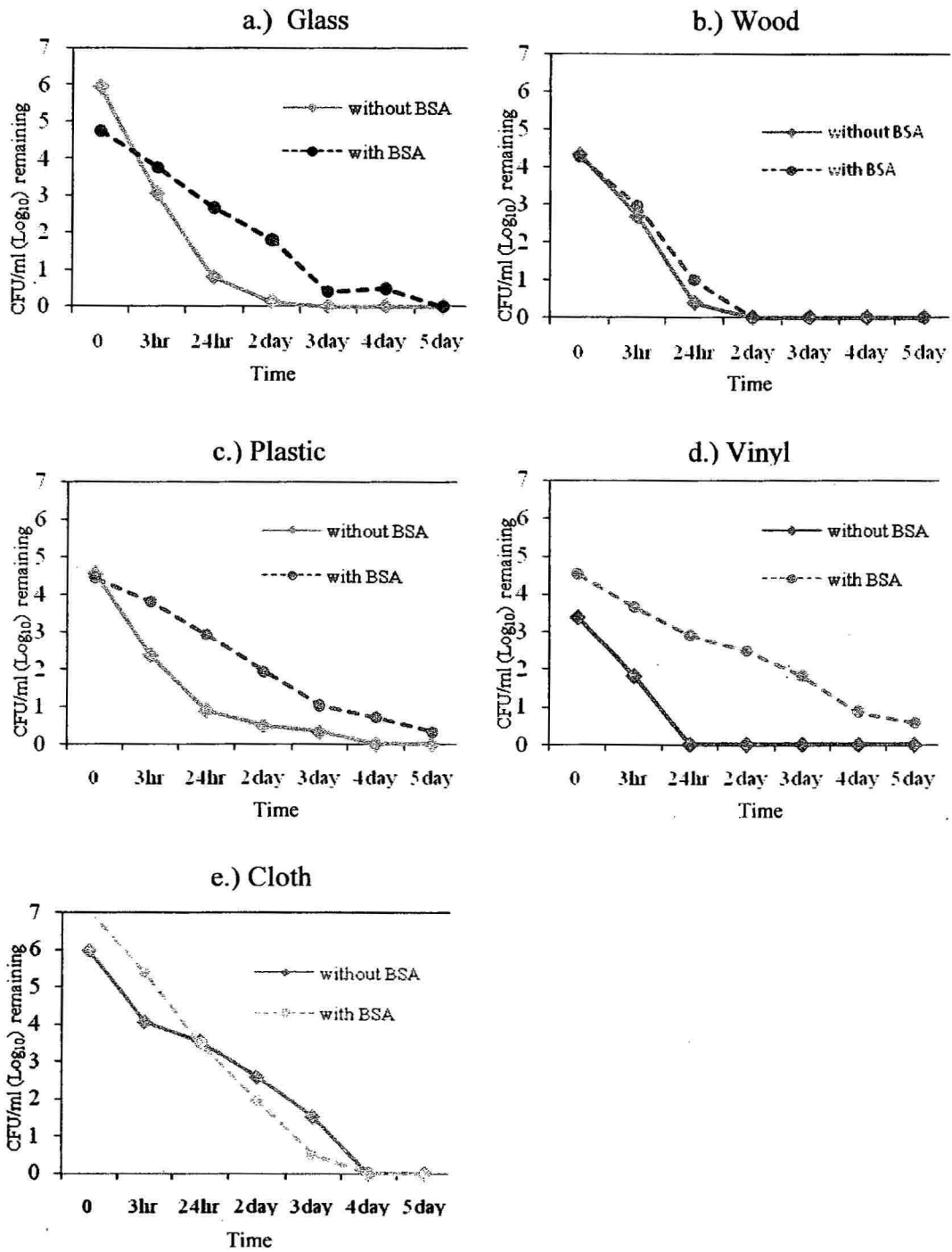


Figure 3: Average concentrations of methicillin-resistant *Staphylococcus aureus* (Log<sub>10</sub> CFU/ml) over time with and without bovine serum albumin (BSA) on a.) Glass b.) Wood c.) Plastic d.) Vinyl e.) Cloth.

Table 2: Means of methicillin-resistant *Staphylococcus aureus* (CFU/ml) with and without bovine serum albumin (BSA) on each surface type.

Surface Type	Mean CFU/ml ( $\log_{10}$ ) with and without BSA ( $\pm 1$ S. E.)	
	with BSA	without BSA
Glass ( $p < .001$ )	1.98 ( $\pm .08$ )	1.42 ( $\pm .08$ )
Wood ( $p < .001$ )	1.18 ( $\pm .08$ )	0.98 ( $\pm .08$ )
Plastic ( $p < .001$ )	2.17 ( $\pm .08$ )	1.18 ( $\pm .08$ )
Vinyl ( $p < .001$ )	2.40 ( $\pm .08$ )	0.74 ( $\pm .08$ )
Cloth ( $p = .023$ )	2.62 ( $\pm .08$ )	2.53 ( $\pm .08$ )

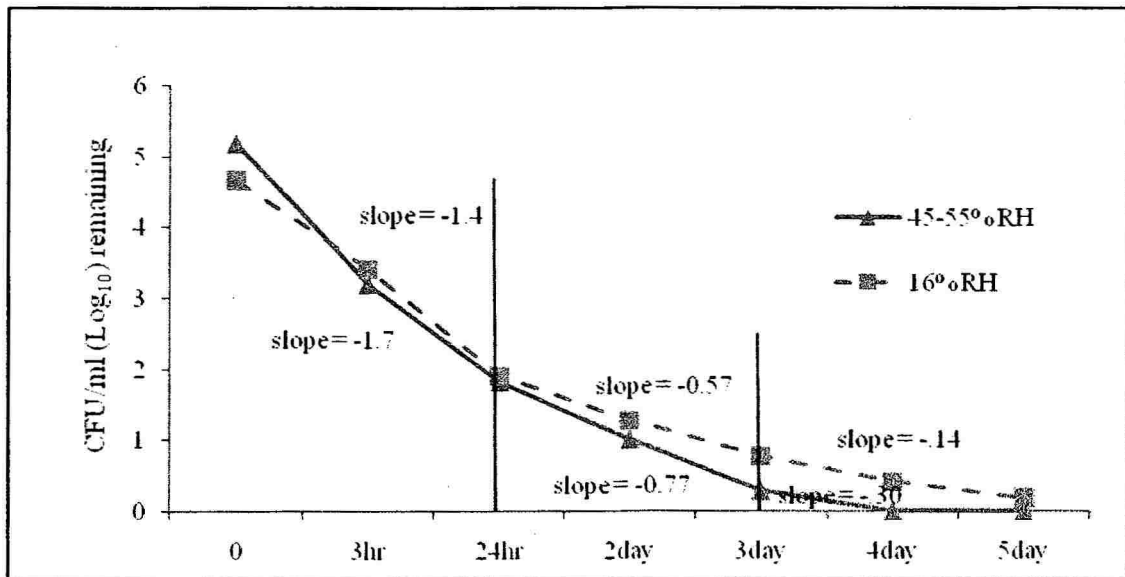


Figure 4: Overall average concentration of methicillin-resistant *Staphylococcus aureus* ( $\log_{10}$  CFU/ml) over time with 45-55% and 16% relative humidity (RH).

Table 3: Means of methicillin-resistant *Staphylococcus aureus* (CFU/ml) with relative humidity (RH) of 45-55% or ambient (16%) on significant surface types.

Surface Type	Mean CFU/ml ( $\log_{10}$ ) ( $\pm 1$ S. E.)	
	45-55% RH	16% RH
Plastic ( $p < .001$ )	1.34 ( $\pm .08$ )	1.97 ( $\pm .08$ )
Vinyl ( $p < .001$ )	1.39 ( $\pm .08$ )	1.76 ( $\pm .08$ )

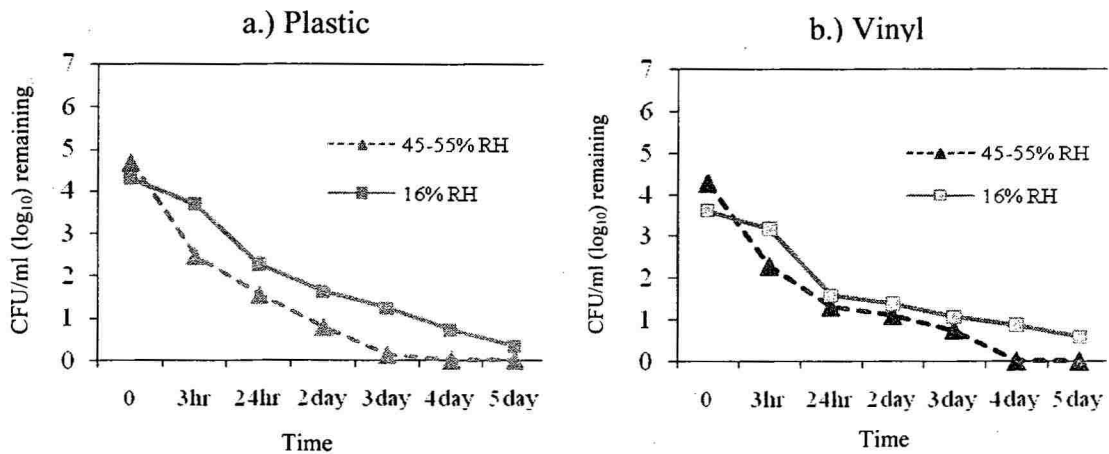


Figure 5: Average concentrations of methicillin-resistant *Staphylococcus aureus* (Log<sub>10</sub> CFU/ml) over time with 45-55% and 16% relative humidity (RH) on a.) Plastic b.) Vinyl.

Table 4: Average concentration of methicillin-resistant *Staphylococcus aureus* (CFU/plate) on replicate organism detection and counting (RODAC) contact samples over time on five surface types at two relative humidities (RH) with and without bovine serum albumin (BSA) (DL = 1 CFU; TNTC = too numerous to count).

Surface type	RH (%)	BSA	Average Concentration (CFU/plate)						
			Time 0	3 hrs	24 hrs	2 day	3 day	4 day	5 day
Glass n=16	45-55%	-	TNTC	TNTC	16.8	2.0	<DL	<DL	<DL
		+	TNTC	TNTC	TNTC	X <sup>1</sup>	1.0	1.0	<DL
	16%	-	TNTC	TNTC	6.0	<DL	<DL	<DL	<DL
		+	TNTC	TNTC	112.5	66.8	7.8	15.3	2.5
Wood n=16	45-55%	-	TNTC	TNTC	<DL	<DL	<DL	<DL	<DL
		+	TNTC	TNTC	1.0	<DL	<DL	<DL	<DL
	16%	-	TNTC	35.3	<DL	<DL	<DL	<DL	<DL
		+	TNTC	TNTC	4.8	<DL	<DL	<DL	<DL
Plastic n=16	45-55%	-	TNTC	156.5	<DL	<DL	<DL	<DL	<DL
		+	TNTC	TNTC	TNTC	64.0	1.3	<DL	<DL
	16%	-	TNTC	TNTC	22.0	1.8	2.0	<DL	<DL
		+	TNTC	TNTC	TNTC	164.5	59.3	56.3	4.8
Vinyl n=16	45-55%	-	TNTC	TNTC	<DL	<DL	<DL	<DL	<DL
		+	TNTC	TNTC	TNTC	125.3	34.0	1.0	<DL
	16%	-	TNTC	TNTC	<DL	<DL	<DL	<DL	<DL
		+	TNTC	TNTC	TNTC	TNTC	TNTC	57.8	16.5

<sup>1</sup> 2 measurements at TNTC, 1 measurement at 25CFU/plate, 1 measurement at 19CFU/plate

## CHAPTER 6

### DISCUSSION

#### Surface Differences

There was a significant difference ( $p < .001$ ) in survival time among the different surface types; therefore null hypothesis 1 is rejected. Viable MRSA cells survived the longest on plastic and vinyl surfaces. This may be a result of the physical contours of these surfaces. At the macroscopic level they appear smooth, yet at the microscopic level there are deep crevasses which may allow cells to sequester with protection from dehydration and colonize. Conversely, the smoother surfaces offer little protection from drying.

This finding is of particular relevance to childcare facilities and homes with children, in that many toys are made from plastic and vinyl. Plastic toys are commonly found in childcare facilities and in homes, and children often share toys. This then results in the toys serving as vehicles for transmission of bacterial cells between children. Vinyl flooring is also common in childcare facilities where children may crawl or sit, posing risk for transmission. Infections in children can quickly develop to serious disease. MRSA infections also result in higher mortality in the elderly. Plastic surfaces such as hand rails or bed rails in assisted living facilities, long term care facilities, or any environment in which the elderly are housed could serve as vehicles for transmission. Furthermore, plastic and vinyl surfaces are present in a number of settings associated

with vulnerable populations such as sporting facilities, hospitals, prisons, and communal living facilities. With viable organisms present on plastic and vinyl for five days or longer, the risk of transmission from that contaminated surface is highly plausible.

In contrast, the survival of MRSA as measured by CFU/ml was less on smooth surfaces which offered the bacteria little protection from desiccation. This finding can be applied to settings in which transmission is more likely to occur. For example, counter tops and bed rails in hospitals and long term care facilities, benches and equipment in sporting facilities and communal living arrangements, flooring and sleeping mats in childcare facilities could be constructed of smooth materials to minimize the survival time of MRSA and ultimately decrease incidence rates of disease.

The lowest survivability of MRSA as measured by CFU/ml was found on wood surfaces. This was surprising as wood is not a smooth, hard surface. It is likely that the swabbing technique used to retrieve the cells from the surfaces over time was not able to effectively remove cells from the deep crevasses that were present in the wood. Additionally, wood is porous and MRSA cells may have dispersed throughout the wood beneath the surface, again making it difficult to retrieve the cells with the swab. Although the likelihood of transmission of MRSA may be lessened for wood surfaces compared to the other surfaces studied in this research, proper precaution should still be observed when installing wooden surfaces in facilities used by vulnerable populations.

Null hypothesis 2 cannot be rejected, as survival time of MRSA was not significantly higher on cloth. The greatest number of CFU/ml overall were found on the flannel cloth. However, no viable bacteria remained on day 4. The MRSA suspension was pipetted onto the flannel cloth and dispersed as much as possible, yet often the liquid was

absorbed onto the fabric at contact. On the other surfaces the liquid remained as a pool which was then dispersed across the designated area. The rapid absorption of the liquid on the flannel cloth may have forced crowding of the cells, resulting in competition that caused cell death, ultimately resulting in fewer viable bacteria. It is also possible that the hand stomaching method used to release the MRSA cells into suspension may have contributed to the underestimation of CFU/ml. Stomaching is very effective at releasing the cells from the porous cloth, but a larger volume of diluent is required than that used with the swab technique. Although the amount of liquid is accounted for in the calculations, it may have resulted in an underestimation of the concentration of CFU/ml.

Regardless of possible methodology flaws in sampling and processing, it is alarming that such a high percentage of MRSA cells were able to persist on cloth for up to three days. This is more than enough time to transmit the bacteria. Additionally, Oie et al. (2004) observed that porous materials, under an electron microscope, remained colonized with MRSA even after disinfection. This raises concern about clothing and accessory items worn in healthcare settings. Often times cotton laboratory coats are worn by healthcare personnel as they move from one room to the next, and are worn for consecutive days without laundering. Ties are also worn by some male healthcare personnel and are unlikely to be laundered in between each wear. Such items can aid in transmission of bacteria. Some countries in the United Kingdom have banned the use of laboratory coats, ties, and even clothing on the arms that go below the elbow in an effort to decrease the transmission of microorganisms and minimize hospital acquired infections (Turaga & Bhagavatula, 2008). Perhaps the same precautionary measures should be employed in the U.S. where the incidence of MRSA is currently on the rise.



Other cloth surfaces such as children's toys/stuffed animals, bed sheets, curtains, sleeping cots, and stretchers can serve as potential reservoirs for transmission. This is especially important for vulnerable populations such as children, the elderly and immunocompromised individuals in which infections can result in serious illness and increased morbidity and mortality.

### The Effect of BSA

The addition of BSA had a significant positive correlation ( $p < .001$ ), enabling longer MRSA survival times. Therefore, null hypothesis 3 is rejected. There was also a significant interaction effect with BSA and surface type ( $p < .001$ ). One potential explanation is that the BSA aided in the adherence to the physical contours of the surfaces. Makison and Swan (2006) found that bacterial cells aggregated within the BSA, and collected at the edges of BSA flakes as drying occurred. Survival time may increase further if the surface type is such that allows for greater adhesion of proteinaceous mediums.

Another explanation is that the BSA offered the cells protection from dehydration. This is important as it is likely the same effect a human protein or bodily fluid would have, essentially allowing the bacteria to survive on the surface for a greater length of time. Tolba et al. (2007) found that the addition of actual human fluid in the form of blood and pus enhanced survival from 4 hours up to 13 days where the experiment was terminated. It is also quite likely that bacteria would be deposited on surfaces with some sort of organic matter (e.g. mucus, saliva) offering protection from desiccation.

## The Effect of Humidity

Previous research has indicated that increases in RH have a negative correlation with bacterial survival (Makison & Swan, 2005; Arundel et al., 1986; Wilkoff et al., 1969). However, there is a gap in the literature when it comes to extremely low RH, such as those in the teens which are quite common in the Las Vegas Valley. It was initially thought that the arid climate would hinder bacterial survivability by quickening dehydration, yet that was not the case. The overall mean in regard to humidity was significantly higher at 16% RH. Null hypothesis 4 is rejected as there was a significant difference ( $p=.002$ ) in survival of MRSA in regard to humidity. Though differences were not significant on all surfaces, bacterial survival was significantly higher at 16% than at 45-55% RH on plastic and vinyl surfaces.

This finding raises questions about the incidence rates of MRSA infections in differing geographical locations. Extremely low RH is common in the Las Vegas Valley, but many other areas of the country the RH remains at a higher percentage. This poses the question as to whether or not areas of moderate to high RH have lower incidence rates of MRSA infection than do areas with lower RH. An epidemiological study is necessary to answer this question.

Arundel et al. (1986) suggest that indoor environments should be kept at 40 to 60% RH to minimize adverse health effects from microbial contamination. Results of the current study compliment their findings.

It is important to note that viable MRSA cells were able to survive for a day or longer on all surfaces, regardless of conditions. In many situations this may be long enough to transmit the bacteria. Even if the organism does not cause immediate infection,

colonization may occur from the contact, and colonization increases the risk of infection fourfold (Furlow, 2009). Consequently, proper disinfection and precautionary measures are vital.

Effective disinfection is an important factor in the prevention of illness. When conducting disinfection it is important to follow the directions on the packaging carefully. Disinfectants such as chlorine bleach solutions are effective at disinfection, but require dirt and soil to be removed from surfaces prior to use to remain effective. Quaternary ammonium compounds and 10% bleach solutions are effective at inactivating MRSA on clean surfaces (CDC, 2008b). Disinfectant cleaning solutions both clean and disinfect in one step. Use of such solutions can be beneficial on surfaces such as vinyl flooring, wooden benches, cloth window treatments, or plastic toys in settings associated with vulnerable populations such as childcare, long term care, and sporting facilities.

### RODAC Contact Sample Results

RODAC contact samples are used quite frequently in healthcare facilities to determine if microbial contamination is present. The use of contact sampling is advantageous because of the long shelf life of the RODAC plates, low cost, consistent and precise recovery, and ease of use by personnel without extensive training (Brummer, 1976). In this study, results from the RODAC contact samples were used as a qualitative method of sampling rather than a quantitative method, as often times the concentration of MRSA CFU/plate were too numerous to count (TNTC). The swab sampling method allowed for quantification when the contact samples did not. Although, results of the contact samples mimicked the results of the swab sampling method. The concentration

of MRSA was greatest with the addition of BSA as well as at the ambient RH (16%) as measured by CFU/plate. Additionally, MRSA persisted on vinyl and plastic at 5 days, while the rate of decline was greatest on wood. Interestingly, in the contact sample results MRSA survival also persisted on glass at five days.

### Research Limitations

There are possible limitations with the sampling methods used on some surfaces. As previously discussed, the swabbing method may not have been successful at removing all bacteria from the deep crevasses and pores present in the wood surfaces and the hand stomaching method may have diluted the cells in suspension. This may have resulted in an underestimation of the CFU/ml surviving on these surfaces.

Previous research has found that MRSA can persist in the environment for as long as seven months. The current study was terminated after 5 days because there was minimal growth remaining as measured by MRSA CFU/ml. With the addition of BSA viable CFU/ml remained at five days on vinyl and plastic, yet not on other surfaces. One possibility as to why survival time could not be replicated may be a result of the particular MRSA strain used (ATCC #43300). This is a laboratory strain that is most often grown under optimal conditions. It may be that this particular strain does not survive well under the specific conditions of this research as compared to other clinical or community strains of MRSA. It is possible that wild-type strains encounter far more obstacles to survival, enabling them to become more resistant to environmental stresses of temperature and relative humidity. A comparison study is necessary to determine strain survival variability.

## CHAPTER 7

### CONCLUSION

Since the advent of antibiotics, the death toll from infectious diseases has decreased dramatically. However, genetic mutations have enabled microorganisms to become resistant to many antibiotic therapies. The spread of antibiotic resistant bacteria has recurrently been a concern in clinical settings, but more recently has become a concern in community settings. The spread of resistant bacteria throughout the community is an essential public health issue, as such infections are often more difficult to treat and result in higher morbidity and mortality rates.

MRSA is one antibiotic resistant organism that is of particular concern. The numbers of infections are increasing in epidemic proportion, accounting for 2% of all *S. aureus* infections in 1974 and increasing to 64% in 2005 (Klebens et al., 2007). The length of hospital stay and cost of MRSA infections are double that of non-MRSA infections (Elixhauser & Steiner, 2007). Claiming the lives of over 18,000 individuals in 2005 alone, MRSA has garnered a great deal of public fear (Klebens et al., 2007). Additionally, vulnerable populations such as children, the elderly, athletes, prisoners, military personnel, blacks and the immunocompromised are disproportionately affected (CDC, 2005; Klebens et al., 2007).

Contamination of surfaces with MRSA is a significant public health concern, as it may result in transmission of the bacteria and have negative health consequences. The

literature on the survivability of MRSA on surfaces is limited, although previous data have found that these organisms can persist on dry surfaces for up to seven months (Kramer et al., 2006).

This study was designed to evaluate the variability of survival of MRSA on five environmental surface materials; glass, wood, vinyl, plastic, and cloth. The effects of RH and BSA, a proteinaceous serum, on MRSA survivability were also examined. Results showed that there was a significant difference amongst the surfaces, with MRSA surviving on plastic and vinyl for the longest amount of time, and on wood the least. This is likely due to the physical contours of the surface; plastic and vinyl provide deep niches in which the bacteria can sequester and colonize, while wood surfaces had very small narrow grooves which may have made retrieval by swabbing inefficient. The overall greatest concentration of CFU/ml remained on cloth, however culturable MRSA did not persist past three days. However, the retrieval method for cloth may underestimate the amount of viable bacteria that remained. The addition of BSA enabled MRSA to survive significantly longer than when the protein serum was not present. It is likely that BSA aided in adherence to the surface and offered protection from dehydration, allowing longer survival times. When surfaces were stored in 45-55% RH the survival of MRSA was significantly less than when they were stored at 16% RH; the latter being a regular occurrence in the Las Vegas Valley. Findings from this study compliment a previous suggestion of keeping indoor environments between 40 and 60% RH to minimize adverse health effects from microorganisms (Arundel et al., 1986).

Information gathered from this study indicates that viable MRSA bacteria can remain on surfaces for days; likely serving as a reservoir for transmission. The best way to

control the MRSA epidemic is to prevent transmission. The most logical and effective means include methods of primary prevention such as proper hand washing, keeping wounds covered, avoiding contact with others wounds, showering after athletic workouts, not sharing personal items such as towels or razors, and asking healthcare professionals to wash their hands before they come in contact with you (CDC, 2005). Effective disinfection methods should also be employed for all surfaces, especially those surfaces in areas with vulnerable populations. Disinfection can be achieved with most over the counter products found in grocery and retail stores provided that the instructions are accurately followed (CDC, 2008a). Also, new research has shown that exposure to visible blue light, not of the harmful UV spectrum, is effective at inactivating MRSA (Maclean et al., 2008; Enwemeka et al., 2008). Further research is needed, yet this may have positive implications on methods of effective surface disinfection.

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