Evaluation of Sani-24 Surface Sampling Methods for Prevention of Recontamination of Staphylococcus aureus and MRSA on High-touch Durable Hospital Surfaces and Equipment

Yasmine Sharifai

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Evaluation of Sani-24 Surface Sampling Methods for Prevention of Recontamination of
Staphylococcus aureus and MRSA on High-touch Durable Hospital Surfaces and Equipment

by

Yasmine Sharifai

Under the Direction of Lisa Casanova, PhD

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Masters of Science

in the College of Arts and Sciences

Georgia State University

2022
Abstract

Hospital-acquired infections is a major public health crisis in the United States. The burden on healthcare workers to eliminate recontamination through repeated cleaning has highlighted the need for continuously active disinfectants against common healthcare pathogens. The study aims to identify proper solutions and methods for surface sampling to detect contamination after disinfecting with Sani-24, a continuous active disinfectant. The sampling solutions and methods tested this study include: Neutralizing Buffer (sodium monopotassium, sodium thiosulfate, aryl sulfonate complex), Eluent (PBS+0.01 % Tween 80), and Cellulose Sponge Stick recovery. When testing MRSA exposure to neutralizing buffer, eluent, and cellulose, no relationship between the bacterial log reduction and time was detected. This showed that the proposed surface sampling methods did not kill the bacteria of interest throughout an extend time, as intended.
Evaluation of Sani-24 Surface Sampling Methods for Prevention of Recontamination of Staphylococcus aureus and MRSA on High-touch Durable Hospital Surfaces and Equipment

By

Yasmine Sharifai

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Electronic Version Approved:

Office of Graduate Services
College of Arts and Sciences
Georgia State University
April 2022
ACKNOWLEDGMENTS

I would like to first thank Dr. Lisa Casanova and Dr. Christine Stauber for providing me with this opportunity, continuous guidance, and patience throughout this journey. I also would like to thank the graduate students in the lab who offered their time and guidance to conduct the experiments. Finally, I would like to thank my family and friends for the most patience and support. I will be forever grateful for everyone who allowed my academic accomplishments possible.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS .................................................................................................................. V

1. INTRODUCTION .......................................................................................................................... 4

2. REVIEW OF LITERATURE ........................................................................................................... 6

2.2 BRIEF HISTORY OF DISINFECTANTS AND STERILIZATION ........................................ 6

   2.1.1 Heat Sterilization ................................................................................................................. 6

   2.1.2 Chemical Sterilization ......................................................................................................... 7

   2.1.3 Radiation Sterilization ......................................................................................................... 7

2.2 HYDROGEN PEROXIDE ........................................................................................................... 8

   2.2.1 Background and Uses of Hydrogen Peroxide ................................................................. 8

   2.2.2 Mechanism ........................................................................................................................ 9

   2.2.3 Advantages of Hydrogen Peroxide .................................................................................. 10

   2.2.4 Synergism of Hydrogen Peroxide .................................................................................... 11

   2.2.5 Vapor-Phase Hydrogen peroxide ..................................................................................... 13

   2.2.6 Uses of Hydrogen Peroxide ............................................................................................. 14

2.3 CONTINUOUS ACTIVE DISINFECTION ............................................................................... 15

2.4 METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) .................................. 17

   2.4.2 MecA Gene ....................................................................................................................... 18

   2.4.3 Transmission of MRSA ..................................................................................................... 18

2.5 SAMPLING AND DETECTION METHODOLOGY ................................................................. 18

   2.5.1 Cellulose Sponge Wipe Process ..................................................................................... 19
2.5.2 Neutralizing buffer ................................................................. 19
2.5.3 Detection: Baird-Parker Agar with Broth Enhancement ................. 19

3. METHODS AND PROCEDURES ....................................................... 21

3.1 Baird-Parker Agar ......................................................................... 21
3.2 Tryptic Soy Broth Dilution Tubes .................................................. 21
3.3 PROCEDURES .............................................................................. 21
    3.3.1 Test to Expose Bacteria to Neutralizing Buffer .......................... 22
    3.3.2 Test to Expose Bacteria to Sani-24 Residual and Neutralizing Buffer .... 22
    3.3.3 Test to Expose Bacteria to Sani-24 Residual, Neutralizing Buffer, and Eluent .... 23
    3.3.4 Recovery of Bacteria from Sponge Sticks .................................... 24
    3.3.5 Statistical Analysis .................................................................. 25

4. RESULTS .......................................................................................... 26

4.1 MRSA Exposure to Neutralizing Buffer ......................................... 26
4.2 MRSA Exposure Sani-24 Residual and Neutralizing Buffer ............. 27
4.3 MRSA Exposure to Sani-24 Residual, Neutralizing Buffer, and Eluent .. 28
4.4 Recovery of MRSA from Sponge Sticks .......................................... 30

5 DISCUSSION AND CONCLUSION .................................................. 32

5.3 DISCUSSION ................................................................................. 32
5.4 CHALLENGES .............................................................................. 34
5.5 CONCLUSION ............................................................................... 34

WORKS CITED .................................................................................. 36

6. TABLES ............................................................................................ 42
7. FIGURES ........................................................................................................... 43
1. INTRODUCTION

Hospital-acquired infections (HAIs) are classified as infections in hospitalized patients that are unrelated to the original illness that brings the patients to the hospital (Revelas, 2012). HAIs contribute to morbidity, mortality, increased cost, and length of hospital stay. According to the CDC, 1 in 31 hospital patients hospital acquired infections, which contributes to billions of dollars in added expenses to the United States healthcare system (2022). Not only do HAIs have a financial effect, but it also leaves many with long term health problems.

It is estimated that about one half of hospital patients receive antimicrobial medication on any given day. HAIs have become increasingly more resistant to antibiotics. The CDC began a campaign to prevent healthcare-associated infections through surveillance, research, and newly developed strategies ("Methicillin-resistant Staphylococcus aureus ", 2019).

Environmental contamination on porous and nonporous hospital surfaces is an important factor contributing to hospital-acquired infections (William A. Rutala, Gergen, Sickbert-Bennett, Anderson, & Weber, 2019). The patient’s environment allows transmission directly from patient to patient and from patients to healthcare workers to other patients. The disinfection of high-touch surfaces is crucial for reducing HAIs. Typically, environmental cleaning involves a physical action of cleaning and application of disinfectant.

A major disadvantage to many surface disinfectants is that they have no residual that can protect against continual recontamination of a disinfected surface. It is very difficult for hospital staff to continuously disinfect high-touch surfaces, while also following manufacturer’s recommendations (Boyce, 2016). Since pathogens such as Clostridium difficile and Methicillin-resistant Staphylococcus aureus (MRSA) are shed by patients and possibly staff, repeated
contamination is inevitable. Efforts to improve disinfection techniques are needed. The goal of this work is to evaluate proper surface sampling methods to test a continuous disinfectant that leaves residual that provides bactericidal properties for a prolonged time. As a foundation for these studies, evaluation of candidate methods for these studies is necessary.

The study aims to identify proper fluid solutions and surface recovery methods for sampling for use in future studies that will evaluate the efficacy of Sani-24, a continuous surface disinfectant. We are proposing the following questions: (1) Does the selected neutralizing buffer affect the concentration of bacteria over time? (2) Does the selected neutralizing buffer effectively neutralize Sani-24 for a prolonged time? (3) Does the eluent promote bacterial growth or die off when used with the neutralizer buffer and Sani-24 over a prolonged time? (4) Is the sponge stick recovery method efficient for surface sampling?
2. REVIEW OF LITERATURE

2.2 Brief History of Disinfectants and Sterilization

A major issue for healthcare professionals and hospitals is the contamination of pathogens (Mohapatra, 2017). It is important to understand different forms of sterilization and disinfection throughout history to develop effective methods today. Disinfection is defined as “the process of complete elimination of vegetative forms of microorganisms except the bacterial spores from inanimate objects”.

Disinfection and sterilization have been observed and documented from ancient times (Mohapatra, 2017). Sterilization is the “process of complete elimination or destruction of all forms of microbial life (i.e., both vegetative and spore forms), which is carried out by various physical and chemical methods”. In early civilizations drying and salting foods was practiced as a form of preservation (Speth, 2017). It is extremely likely that Neanderthals boiled water and food in perishable hide or a birch bark container because they did not yet have stone-pots or fireproof containers.

2.1.1 Heat Sterilization

John Tyndall invented the process called Tyndallization, which used multiple heating and cooling treatments to eliminate bacterial spores (Aardal, 2015). This is effective because the endospores that can survive the initial heat treatment become vegetative cells during the cooling period, then are effectively killed during the second heat treatment.

Another form of important sterilization is the autoclave. Since there are many microorganisms that can contaminate healthcare instruments the autoclave is useful to sterilize
many items used in hospitals. The first autoclave was invented by Charles Chamberland to fulfill Louis Pasteur’s need for a sterilization process that used temperatures higher than 100°C (Oyawale & Olaoye, 2007).

2.1.2 Chemical Sterilization

The early chemical disinfectants recorded were vinegar, wine, copper, and silver. Many other substances such as mercury, copper sulphate, and sodium permanganate have been used throughout time (Hugo, 1995). Hypochlorites, including household bleach, are the most used chlorine disinfectants and have been used for over 100 years (W A Rutala & Weber, 1997). Hypochlorites used in hospitals as a disinfectant on environmental surfaces such as bed rails, beside tables, and furniture. The general group of Hypochlorites are even used to disinfect water in low level concentrations. These products have many benefits such as inexpensive, fast acting, broad spectrum of antimicrobial activity, and are unaffected by water hardness. However, direct skin exposure to sodium hypochlorite, commonly known as household bleach, can cause irritation, pain, swelling, and respiratory irritation.

2.1.3 Radiation Sterilization

Radiation sterilization was discovered within years after the discovery of radioactivity (Hugo, 1995). Radiation sterilization uses ionizing radiation to eliminate all living organism on the item (Hammad, 2008). This process used primary for the sterilization of health care equipment. High energy X-rays for sterilizing health care equipment was founded in the 1960s but was not readily available until the 1990s. An important factor is verifying these machines are properly sterilizing, which lead to the International Organization for Standardization (ISO)
creating quality assurance and control processes. Another challenge is that each microorganism has developed different levels of resistance to radiation sterilization. Each species is affected by the amount of direct damage, type and length of radiation, ability of the cell to repair damage, and the intra- and extracellular environments influence. The inconsistently of radiation sterilization levels necessary and high cost, makes this form of sterilization less common.

2.2 Hydrogen Peroxide

2.2.1 Background and Uses of Hydrogen Peroxide

Hydrogen peroxide is an important chemical with a high demand for many uses (Block, 2001). Hydrogen peroxide is naturally present in milk and honey, which assists in preventing spoilage (Abdollahi & Hosseini, 2014). Louis-Jacques Thenard discovered hydrogen peroxide (H₂O₂) through barium peroxide and nitric acid’s reaction. Later, hydrogen peroxide began production with hydrochloric acid and then the addition of sulfuric acid to precipitate sulfuric acid (Jones, 1999). Thenard then created the first commercial manufacture of hydrogen peroxide, and this technique was still employed until the middle of the 20th century. However, there were some disadvantages to this process. The market was small for hydrogen peroxide because the production cost was extremely high. Also, there were a substantial number of impurities found in the product while using this process. In 1853, Meidinger discovered that hydrogen peroxide could be formed electrolytically from sulfuric acid. This was very important because this discovery led to many other notable developments to produce large amounts of 100% H₂O₂.

Within the past 20 years there has been an increase in demand for hydrogen peroxide because many findings have revealed its versatility (Block, 2001). In 1968, Yoshpe-Purer and
Eylan found that hydrogen peroxide in low concentrations can be used in drinking water (Block, 2001; Robinson & D'Amico, 2021). In 1972, Naguib and Hussein discovered that 0.1% hydrogen peroxide for 30 minutes at 54°C reduced total bacterial count in raw milk by 99.999%, which was later very important in controlling for bacteria like *Listeria monocytogenes* in high-moisture cheeses. In 1978, Rosenswig found that 0.03% hydrogen peroxide killed 1 million colony-forming units of seven strains of bacteria overnight.

Using hydrogen peroxide at 10-30% concentration has proven to be a promising sporicidal agent as well as could for health (Urban, Rath, & Radtke, 2019). Not only has hydrogen peroxide shown antibacterial affects, but it can be used as a chemical to induce a hemostasis effect to significantly lower blood loss (Urban et al., 2019). While the hemostatic mechanisms are not exactly clear, it is thought be due to the blood platelets exposed to collagen and arachidonic acid which stimulates a series of pathways. If hydrogen peroxide is diluted using clean equipment and deionized water, it remains extremely stable and decomposition only effects contaminated materials, rather than the hydrogen peroxide itself (Block, 2001).

Depending on the concentration, hydrogen peroxide can lead to irritation and whitening of the skin with even just brief contact (Jones, 1999). Long dermal contact can cause burns and injury. 3% hydrogen peroxide can cause respiratory irritation and anything higher than 10% can cause severe pulmonary irritation. Therefore, it is important for hydrogen peroxide to be used safely, especially when used commercially (ATSDR, 2002).

2.2.2 Mechanism
Since hydrogen peroxide is a normal product of cellular metabolism in tissues, it can naturally protect us from pathogenic microorganisms, specifically in the mouth (Block, 2001; Klebanoff, Clem, & Luebke, 1966). It is a powerful oxidant and inhibits the growth of many organisms. Thiocyanate (SCN\(^-\)) and peroxidase are important for antimicrobial activity of hydrogen peroxide \((H_2O_2)\), specifically in saliva (Klebanoff et al., 1966).

\[
H_2O_2 + SCN^- \rightarrow OSCN^- + H_2O
\]

Hydrogen peroxide also works as germicide, killing microorganisms that gain entrance into the blood stream (Bergendi, Beneš, Ďuračková, & Ferenčik, 1999). Within the cells, hydrogen peroxide is produced by the reduction of oxygen. This is done through a series of enzymatic steps that produce not only hydrogen peroxide, but a superoxide anion radical and a hydroxyl radical (Linley, Denyer, McDonnell, Simons, & Maillard, 2012). It is also believed that metals such as iron \((\text{Fe}^{2+})\) and copper \((\text{Cu}^+)\) that are present within the body, leads to the production of more highly toxic hydroxyl radicals. This is referred to as Fenton chemistry. The hydroxyl radicals are extremely potent and believed to do the actual damage to bacteria (Block, 2001). This is what allows phagocytes to absorb the microorganism and use the produced hydroxyl radical to kill bacteria.

### 2.2.3 Advantages of Hydrogen Peroxide

A major advantage of hydrogen peroxide is the broad-spectrum activity, including bacterial endospores (Linley et al., 2012). It is active against a range of bacteria, yeasts, and viruses (Block, 2001). Anaerobic bacteria, such as *Escherichia coli*, are especially sensitive
because they do not produce a catalase to break down peroxide. A tested .05% accelerated H₂O₂ product showed bactericidal activity of 7 different species of vegetative bacteria (Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella choleraesuis, MRSA, E. coli, VRE, and Acinetobacter baumannii) within 1 minute at 20°C (Omidbakhsh & Sattar, 2006). The .05% accelerated H₂O₂ proved virucidal activity in 10 minutes at 20°C. The same .05% accelerated H₂O₂ proved a > 6.8 log₁₀ reduction of Mycobacterium bovis, a mycobacterium, and 5.47 log₁₀ reduction of Trichophyton mentagrophytes, a fungus, to undetectable levels after 5 minutes at 20°C. While it is less rapid against yeasts, certain viruses, and bacterial spores, it is a complete sterilant at concentration of 6% (Block, 2001).

Sagripanti & Bonifacino (1996) found that hydrogen peroxide was effective in killing 90% of bacterial spores between pH 2 and pH 10, with little change throughout. However, in the same study, a higher concentration (35%) and temperature (80°C) proved to have an important effect on sporicidal activity (Sagripanti & Bonifacino, 1996). 35% hydrogen peroxide at 80°C for 3 to 9 seconds are now used for aseptic packaging (Block, 2001).

It is also important to note that once completely degraded, H₂O₂ is environmentally non-toxic (Linley et al., 2012). It is naturally found in plants and leaves no residue when degraded in water and oxygen, therefore it does not require any rinsing or wiping (Ölmez & Kretzschmar, 2009).

2.2.4 Synergism of Hydrogen Peroxide
Synergism is defined as the "interaction of discrete agencies, agents, or conditions such that the total effect is greater than the sum of the individual effects" ("Webster's New International Dictionary of the English language," 1959). In 1930, Dittmar and colleagues discovered that *E. coli* and *S. aureus* phenol coefficient’s was increased 100 times when one part cupric and ferric ions were added to 500 parts of hydrogen peroxide (Block, 2001). This indicates that cupric and ferric ions promote the free-radical oxidation of organic compounds with hydrogen peroxide and enforced the idea that the bactericidal action of hydrogen peroxide was from the free-radicals.

In 1976, Bayliss and Waites examined the effect of hydrogen peroxide’s ability to kill bacterial spores with various cations. When *Clostridium bifermantans* was treated with .28 M of hydrogen peroxide at 25°C there was 87% colony formation (Bayliss & Waites, 1976). When just copper cations alone were used 95% of colonies formed. However, when used together only .028% of colonies formed. This 3000-fold reduction indicated that copper increases rate of breakdown of hydrogen peroxide by promoting the free-radical oxidation system.

Many other elements or compounds have found to increase activity of hydrogen peroxide when combined. Ascorbic acid was found to cause a large increase in DNA base damage when added to the Cu-HP system (Block, 2001). It was also found that 99.99% of Bacillus and Clostridium spores were killed when 30s u.v. light irradiation with 1.0g/100 ml of hydrogen peroxide were used together (Bayliss & Waites, 1979). This was a 2000-fold increases in kill rate compared to u.v. light irradiation alone. However, u.v. light is limited to clear products or limited to the surface.
2.2.5 Vapor-Phase Hydrogen peroxide

Unlike ethylene oxide and formaldehyde, vapor-phase hydrogen peroxide falls into the category for non-toxic, non-carcinogenic, and environmentally friendly as sterilant (Block, 2001). It proved to hold the advantage of a short cycle time, low temperature, with comparatively good material compatibility (Fox, Anderson, Otto, Pritchett-Corning, & Whary, 2015). Many bactericidal, sterilant, and sporicidal properties of hydrogen peroxide have been observed. There are two methods to apply vapor-phase hydrogen peroxide (Fox et al., 2015). The first includes a deep vacuum and a heated vaporizer to pull 30-35% liquid concentrated H$_2$O$_2$ and deliver to a sterilization chamber. The second method involves a vacuum system of either low negative or positive pressure. This method is useful for decontamination or large or small atmospheric systems.

In 1990, Klapes and Vesly conducted a study examining vapor-phase hydrogen peroxide to decontaminate inside a centrifuge chamber, using B. subtilis spores. Bacterial sporicidal were examined because they are the most chemical germicidal-resistant form of microbial life. The study found bacterial spores to be consistently killed within 8 minutes (Klapes & Vesley, 1990). It was found that cycle times > 32 minutes allowed less accessible areas to effectively decontaminate. This study also found that vapor-phase hydrogen peroxide was more effective on the rotor that is at 4°C, compared to the gap areas which are between 19°C and 27°C, leading to the assumption that sporicidal activity was greater at lower temperatures. This is believed to be because higher temperatures increase decomposition rate of the gas.
Many factors contribute to a successful cycle of inactivation of spores with hydrogen peroxide vapor. Humidity, vapor concentration, and condensation levels are all important influences on microbial inactivation rate (Unger-Bimczok, Kottke, Hertel, & Rauschnabel, 2008). It was found that greatest rate of inactivation is with near-saturation, high humidity, high concentration, and no contaminants the surface, such as liquids or oils to impede penetration (Block, 2001).

A systematic review of literature indicated that airborne hydrogen peroxide is an effective method of disinfection of hospital inanimate environments (Falahas et al., 2011). It was found in three studies that the airborne hydrogen peroxide eradicated *C. difficile* in hospital practices. Overall, either hydrogen peroxide vapor or dry mist proves is beneficial when attempting to disinfect a hospital environment when added to current cleaning protocols.

2.2.6 Uses of Hydrogen Peroxide

Hydrogen peroxide was not always as popular as it is today. It is considered safe enough for use in foods in many countries (Zhu, Wang, Lu, & Niu, 2017). A large use is for sterilization of aseptically preserved food, such as milk or juices.

An important use for hydrogen peroxide is lower to nosocomial infections, which are hospital-acquired infections (Revelas, 2012). These are classified as unrelated to the original illness that brings the patients to the hospital. This issue is more important than ever because hospitals are growing larger with many people who are already sick and immunocompromised. This leaves people in the hospital for longer periods of time, leaves them sicker on average, and
more likely to readmit (Humayun, Qureshi, Al Roweily, Carig, & Humayun, 2019). Nosocomial infections contribute to morbidity, mortality, increased cost, and length of hospital stay, which creates a large public health issue. Not only is hospital overcrowding an issue, but this leads to the discovery of new microorganisms and an increased bacterial resistance to antibiotics.

In 2019, Humayun et al. conducted a study to determine the efficacy of hydrogen peroxide fumigation for the improvement of disinfection for hospital rooms. They took samples from environmental surfaces, like beds, incubators, instruments, crash carts, tables, telephones, doors, and air conditioners, that were routinely touched and may play a role in the spread of microorganisms (Humayun et al., 2019). The results showed almost no microbial growth after a vaporized hydrogen peroxide fogging treatment was used.

Hydrogen peroxide is also commonly used to control hydrogen sulfide and odors in wastewater. Between 15 and 40 mg/L of H₂O₂ is effective in controlling the production of H₂S, which results in the unwanted odor (Cole, Paul, & Brewer, 1976). Another common use is 35% concentration of hydrogen peroxide for swimming pools and spas (Stratton). Hydrogen peroxide is often used because it is relatively safe in lower concentrations, however it must be used with ultraviolet light and operate 24 hours a day.

2.3 Continuous Active Disinfection

Cleaning and disinfection are limited by the rate of recontamination, especially in hospital settings where patients stay within the room. Many efforts are focused on high-touched surfaces
and recontamination of these surfaces. This leads to the importance of new technologies that administer continuous disinfection properties.

A 2019 preliminary study evaluated an Environmental Protection Agency (EPA) a novel disinfectant that claims to kill microbes on surfaces for at least 24 hours (William A. Rutala et al., 2019). The intent of this product was not to sterilize, but to “clean” surfaces of pathogens to sufficient level to prevent human diseases. The continuously active disinfectant (CAD) results in 3-5 \( \log_{10} \) reduction of epidemiologically important bacterial pathogens (\( S. \) aureus, VRE, \( C. \) auris, carbapenem-resistant \( E. \) coli, antibiotic-sensitive strains of \( E. \) coli, and Enterobacter spp) in 5 minutes over 24 hours. It showed lower reductions in carbapenem-resistant isolates of Enterobacter spp and \( K. \) pneumoniae, and of antibiotic-sensitive \( K. \) pneumoniae. An important finding from this study was a comparison of the mean \( \log_{10} \) reduction to 3 other commonly used disinfectants for \( S. \) aureus. The mean \( \log_{10} \) reductions were: 4.4 for CAD, 0.9 for quaternary ammonium compound with alcohol, 0.2 for improved \( H_2O_2 \), and 0.1 for chlorine, indicating that over the 24-hour tested time the novel CAD disinfectant proved to show the best prolonged disinfecting properties.

In 2021, two methods for continuous disinfection were compared to each other. One application was a CAD against several pathogens with quaternary ammonium disinfectant. The other application was through portable medical equipment to reduce contamination (Redmond et al., 2021). It was found that the quaternary ammonium active disinfectant provided prolonged antimicrobial activity, resulting in \( \geq 5 \log_{10} \) reduction of multiple pathogens with 5-minute exposure, which was consistent with the previous studies findings. However, it showed no
reduction in *Clostridioides difficile* The portable devices itself were found to become contaminated during medical procedures and patient care activities. It was suggested to apply CAD products onto the portable medical devices to combat this issue.

### 2.4 Methicillin-Resistant Staphylococcus aureus (MRSA)

#### 2.4.1 Background of MRSA

MRSA is a type of *Staphylococcus aureus* bacteria that is resistant to multiple antibiotics and often causes skin infection ("Methicillin-resistant Staphylococcus aureus", 2019) While it is common in the community, within healthcare settings causes more severe problems such as bloodstream infections, pneumonia, or surgical site infections. Everyone is at risk for MRSA, but risk increases with activities or places that involve skin-to-skin contact or shared equipment and supplies. Skin with abrasions or incisions is a common site of infection. About 5% of patients in U.S. hospitals carry MRSA on their skin or in their nose. Cleaning hands and body regularly, specifically after exercise; keeping cuts, scrapes, and wounds clean until completely healed; and avoiding shared personal items can all reduce risk of infection.

In 1959, methicillin was created to treat infections due to penicillin-resistant *S. aureus* (Enright et al., 2002). By 1969, it was reported that *S. aureus* had developed a resistance to the entire class of β-lactam antibiotics (methicillin and penicillin) and was identified as MRSA. MRSA is now an international problem, specifically in nursing homes and hospitals. Initially, healthcare-acquired MRSA was only detected, and later community acquired and livestock acquired MRSA have been detected, with constant newer and more virulent strains emerging (Stefani et al., 2012).
2.4.2 MecA Gene

*Mec*A is the gene responsible for the resistance of staphylococci to penicillin-like antibiotics (Saber, Jasni, Jamaluddin, & Ibrahim, 2017). This gene is not observed in other strains of *S. aureus* that are susceptible to these antibiotics. Staphylococcal cassette chromosome *mec* (SCCmec) carries *mecA* on a mobile genetic element (Enright et al., 2002). While the origin of SCCmec is not known, homologues of the gene were found in *S. sciuri* and *S. vitulinus*, just not in the same SCCmec carrier complex (Saber et al., 2017). The SCCmec with the *Mec*A gene encodes for a low-affinity penicillin-binding protein called PBP2a, which β-lactam antibiotics are unable to inhibit (Otto, 2012).

2.4.3 Transmission of MRSA

MRSA spreads by contact with people or items carrying the bacteria. Within the hospital, contaminated objects and services serve as transitional sources of MRSA (Otto, 2012). However, infections originate from patients or staffs carrying MRSA. Many studies have confirmed that *S. aureus* come from the strains that colonize the nose. This is likely because when mucin layers are breached, matrix proteins are exposed, which allow *S. aureus*-binding proteins to interact. Also, Staphylococci are known to be good biofilm formers, which give protection from antibiotics and the nose can be comparable to biofilms. *S. aureus* colonize or form a biofilm in or on human epithelia and are relatively less aggressive, which allows it to remain undetected longer.

2.5 Sampling and Detection Methodology
2.5.1 Cellulose Sponge Wipe Process

A 2017 study compared four types of swabs: cotton, gauze, polyurethane foam (PU foam) and cellulose sponge) to recover four food borne pathogens (*Salmonella, S. aureus, E. coli, L. monocytogenes*) (Keeratipibul et al., 2017). The cellulose sponge proved to have the higher bacterial recovery efficiency of the four. It was also found that swabbing on wet surfaces with PU form or cellulose had a higher efficiency of bacterial recovery, but the lowest on dry surfaces. Cellulose and PU foam had highest percentage recovery of biofilm and on various surfaces.

2.5.2 Neutralizing buffer

A study was conducted to determine the efficacy of Bacto neutralizing buffer (NB) on cetylpyridinium chloride (CPC) on inoculated cut iceberg lettuce (Osman, Janes, Story, Nannapaneni, & Johnson, 2006). CPC is a water-soluble ammonium compound sprayed to reduce *Salmonella* on different types of meats and produce. Bacto is a modified standard buffer to neutralize sanitizers. It is used to detect microbes after food processing equipment surfaces have been sanitized with quaternary ammonium compounds or chlorine. NB contains sodium monopotassium for the buffering properties, sodium thiosulfate to inactivate chlorine compounds, and an aryl sulfonate complex to neutralize the quaternary ammonium effects (DIFCO). The study found that the Neutralizing Buffer solution was successful to quench the killing action of CPC up to 0.4% concentration.

2.5.3 Detection: Baird-Parker Agar with Broth Enhancement
In 1971, Baer et al. conducted a study comparing the efficiency of two enrichment and four plating medias to isolate *S. aureus*. The two broths compared were Trypticase Soy Broth with 10% NaCl (TSBS) and tellurite enrichment broth (TEB). It was found that *S. aureus* performed better with TSBS with all combinations of plating media. Vogel and John agar, egg yolk 100 agar, tellurite polymyxin egg yolk agar, and Baird-Parker medium all proved equally efficient when detecting *S. aureus* (Baer, Gilden, Wienke, & Mellitz, 1971).
3. METHODS AND PROCEDURES

3.1 Baird-Parker Agar

63.0 g of dehydrated product media was suspended 1 L of deionized water and mixed thoroughly until dissolved. Mixture was autoclaved at 121°C for 15 minutes. Once cooled to 45°C to 50°C, 50 mL of Egg Yolk Tellurite Enrichment was aseptically added. Finished product was then poured into petri dish plates and stored at 20°C until use, room temperature.

3.2 Tryptic Soy Both Dilution Tubes

25.0 grams of Bacto Tryptic Soy Broth dehydrated media was suspended in 1 L of deionized water and mixed thoroughly until dissolved. Mixture was autoclaved at 121°C for 15 minutes. Once cooled, 900 µL of broth was allocated to sterile microcentrifuge tubes and stored at 4°C until use.

3.3 Procedures

<table>
<thead>
<tr>
<th>Test</th>
<th>Exposure</th>
<th>Sampling Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test #1</td>
<td>1. Neutralizing Buffer</td>
<td>Dilution series every 10 minutes</td>
</tr>
<tr>
<td>Test #2</td>
<td>1. Neutralizing Buffer 2. Sani-24</td>
<td>Sponge stick recovery Dilution series every 10 minutes</td>
</tr>
</tbody>
</table>
3.3.1 Test to Expose Bacteria to Neutralizing Buffer

1 mL of stock culture (MRSA, ATCC #43300) was thawed and vortexed. 20 mL of neutralizing buffer (NB) (BD product #236210) was placed into a 50 mL centrifuge tube and the 1 mL stock culture was added and vortexed for about 30 seconds. Three 500 μL samples were taken from NB/stock culture mixture and placed into 1.5 mL sterile microcentrifuge tubes. These tubes were designated as Time₀. This procedure was repeated at 10, 20, 30, 40, 50, and 60 minutes. For each 500 μL sample, four 1:10 dilutions were made with 900 μL TSB dilution tubes. 100 μL was pipetted from each dilution and spread onto a Baird-Parker plate. This was done twice for each dilution tube, including the undiluted sample. Plates were incubated with lid down at 37°C for 18-24 hours and 42-48 hours, then counted and recorded. Plates were counted at twice to ensure correct colony count was recorded. The 42-48 hour time period was the final data used for results.

3.3.2 Test to Expose Bacteria to Sani-24 Residual and Neutralizing Buffer

1 mL of stock culture (MRSA, ATCC #43300) was thawed and vortexed. Sterile 10 cm² stainless steel square was wiped with Sani-24 wipe in 3 fluid singular motions: horizontally, vertically, and diagonally. 3M™ Sponge-Stick with 10 mL Neutralizing Buffer (product
#SSL10NB) was used to immediately (10-20 seconds) wipe the square in the same pattern as before. Handle was removed and the sponge was placed into bag and closed securely. Bag was rolled for 2 minutes with rolling pin to squeeze all liquid from sponge. With 5 mL pipette, the sponge was fished out of the bag. The remaining liquid was pipetted into 50 mL centrifuge tube, which was approximately 10mL total. 1 mL of stock culture was added to the 50 mL centrifuge tube and was vortexed well. Three 500 µL samples were taken from mixture and placed into 1.5 mL sterile microcentrifuge tubes. These tubes were designated as Time0. This procedure was repeated at 10, 20, 30, 40, 50, and 60 minutes. For each 500 µL sample, four 1:10 dilutions were made with 900 TSB µL dilution tubes. 100 µL was pipetted from each dilution and spread onto a Baird-Parker plate. This was done twice for each dilution tube, including the undiluted sample. Plates were incubated with lid down at 37ºC for 18-24 hours and 42-48 hours, then counted and recorded.

3.3.3 Test to Expose Bacteria to Sani-24 Residual, Neutralizing Buffer, and Eluent

1 mL of stock culture (MRSA, ATCC #43300) was thawed and vortexed. Sterile 10 cm² stainless steel square was wiped with Sani-24 wipe in 3 fluid singular motions: horizontally, vertically, and diagonally. 3M™ Sponge-Stick with 10 mL Neutralizing Buffer (product #SSL10NB) was used to immediately wipe the square in the same pattern as before. Handle was removed and the sponge was placed into the bag. 20 mL of eluent (PBS+0.01 % Tween 80) was added to the bag and closed securely. Bag was rolled for 2 minutes with rolling pin to squeeze all liquid from sponge. With 5 mL pipette, the sponge was fished out of the bag. The remaining liquid was pipetted into 50 mL centrifuge tube, which was approximately 30 mL total. 1 mL of
stock culture was added to the 50 mL centrifuge tube and was vortexed well. Three 500 µL samples were taken from mixture and placed into 1.5 mL sterile microcentrifuge tubes. These tubes were designated as Time0. This procedure was repeated at 10, 20, 30, 40, 50, and 60 minutes. For each 500 µL sample, four 1:10 dilutions were made with 900 µL TSB dilution tubes. 100 µL was pipetted from each dilution and spread onto a Baird-Parker plate. This was done twice for each dilution tube, including the undiluted sample. Plates were incubated with lid down at 37°C for 18-24 hours and 42-48 hours, then counted and recorded.

3.3.4 Recovery of Bacteria from Sponge Sticks

1 mL of stock culture (MRSA, ATCC #43300) was thawed and centrifuged in a microcentrifuge at 10,000 RPM for 3 minutes. Once completed, the supernatant was drawn off the tube and discarded. 900 µL of phosphate buffered saline (PBS) was added to the tube and vortexed to resuspend the pellet. 100 µL of fetal bovine serum was added to the tube with the PBS and pelleted cells, then vortexed for about 30 seconds. Three Sponge-Sticks with 10 mL Neutralizing Buffer (product #SSL10NB) were removed from each bag. Immediately, 100 µL of bacteria inoculum was pipetted onto each sponge. Sticks were broken off and sponges were placed back into individual bags. 25 mL of eluent (PBS+0.01 % Tween 80) was added to each bag and closed securely. With a rolling pin, remaining liquid was squeezed out of the sponge. This was done for 2 minutes to ensure all liquid was squeezed out of sponge. With a 5 mL pipette, sponge was fished out of the bag. Remaining liquid from each was pipetted into individual 50 mL centrifuge tube, which was about 30-35 mL total. All tubes were centrifuged at 2500 RPM for 20 minutes. Once completed, supernatant was drawn off from each tube without disturbing the pellet. This was done to keep a standard volume to bacteria ratio. Pellet was
resuspended with 10 mL of PBS without tween. Dilution series with each 10 mL tube was done. 100 µL of each sample was pipetted into 900 µL of TSB (dilution #6). Five more dilutions were made. 100 µL was pipetted from each dilution and spread onto a Baird-Parker plate. This was done twice for each dilution tube, including the undiluted sample. Plates were incubated with lid down at 37°C for 18-24 hours and 42-48 hours, then counted and recorded.

3.3.5 Statistical Analysis

SAS Procedure Regression was used to conduct an Analysis of Variance test (ANOVA) test. ANCOVA was used to assess the variation of bacterial growth due to time. This test determined if time (variable) affects the bacterial growth (outcome). Variation was defined as differences in the log reduction across the different time intervals observed. Time was the duration of each interval sampled during the experiment. Survival of MRSA bacteria was determined by calculating \( \log_{10} \left( \frac{N_t}{N_0} \right) \), log reduction. \( N_0 \) is the bacterial CFU/mL at time zero and \( N_t \) is the CFU/mL at various time intervals. Survival of MRSA bacteria when using the sponge stick recovery method was determined by calculating the log reduction of bacteria recovered from the surface with the MRSA bacterial titer. The average percent recovered from all trials was calculated.
4. RESULTS

4.1 MRSA Exposure to Neutralizing Buffer

The first experiment examined survival of MRSA exposed to Neutralizing Buffer over one hour. This length of time was chosen as the maximum that would be allowed to elapse between when a sample is collected and when it is processed. Over 60 minutes, there was <0.5 log reduction of bacteria, and bacterial growth was not apparent during the time period (Figure 2). Overall, there was no significant bacterial growth or die off during the one-hour interval. The average concentration at each time interval can be seen in Figure 1. As seen in Figure 2, time was not a significant (\( p = 0.6421 \)) predictor of survival over 1 hour.

Figure 1. MRSA concentration (\( \log_{10} (N_t) \)) over 1 hour of exposure to neutralizing buffer
Figure 2. MRSA and neutralizing buffer: Log Reduction ($N_t/N_0$) Vs. Time

4.2 MRSA Exposure Sani-24 Residual and Neutralizing Buffer

This experiment examined concentration of MRSA exposed to the neutralizing buffer and Sani-24 residual over one hour (Figure 3). There was minimal change at all time points (Figure 4). Time was not a significant predictor (P-value = 0.4592) of bacterial concentration. There was no apparent growth or die off of bacteria during the one-hour interval.
Figure 3. MRSA concentration ($\log_{10} (N_t)$) over 1 hour of exposure to neutralizing buffer and Sani-24 residual

Figure 4. MRSA, neutralizing buffer, and Sani-24 residual: Log Reduction ($N_t/N_0$) Vs. Time

4.3 MRSA Exposure to Sani-24 Residual, Neutralizing Buffer, and Eluent

This experiment examined bacterial growth of MRSA exposed to the Neutralizing Buffer, Sani-24 residual, and eluent over 60 minutes. Figure 5 showed the mean concentration in $\log_{10}$
(CFU/mL) for each time interval; concentrations varied by approximately 0.5 log over 1 hour. Figure 6 showed the log reduction vs. time, which was not a significant predictor of bacterial concentration ($p = 0.4908$). Overall, there was no significant bacterial growth or die off during the one-hour interval. This experiment showed more variability, which was due to difficulty reading the plates. This issue was further discussed in the challenges section (Section 5.4).

Figure 5. MRSA concentration (over 1 hour of exposure to neutralizing buffer, Sani-24 residual, and eluent)
4.4 Recovery of MRSA from Sponge Sticks

This experiment examined the recovery of MRSA from sponge sticks used for surface sampling. The calculated MRSA bacterial titer was approximately $5.6 \times 10^6$ CFU/mL. The concentration of MRSA recovered from sponge sticks ranged from $2.39 \times 10^6$ CFU/mL to $4.45 \times 10^6$ CFU/mL and was not significantly different ($p= .4908$) between trials. Figure 7 and Table 1 also show recovery and between-trial variation. Figure 8 shows the log reduction of each trial using the bacterial titer. The average percent recovered from all trials was 63.78%, using the MRSA bacterial titer as the denominator. Overall, there was no significant bacterial growth or die off when using the sponge stick method.
Figure 7. Recovery of MRSA from Sponge Sticks: Log concentration ($\log_{10}(N_t)$) vs. bag number

![Graph showing recovery of MRSA from Sponge Sticks with log concentration vs. bag number.]

Figure 8. Recovery of MRSA from Sponge Sticks: Log reduction (\(\log \frac{N_t}{N_0}\)) vs. bag number

![Graph showing recovery of MRSA from Sponge Sticks with log reduction vs. bag number.]

Table 1. Recovery of MRSA from Sponge Stick

<table>
<thead>
<tr>
<th>BAG 1</th>
<th>BAG 2</th>
<th>BAG 3</th>
<th>BAG 4</th>
<th>BAG 5</th>
<th>BAG 6</th>
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<tr>
<td>$\log_{10}(B_N)$</td>
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5 DISCUSSION AND CONCLUSION

5.3 Discussion

The results of these experiments showed that sampling solutions necessary for conducting disinfection experiments- neutralizing buffer and eluent solution- do not result in significant growth or reduction of the target bacterium over an interval of contact with sampling solutions for one hour. Results from the first test, “Test to Expose Bacteria to Neutralizing Buffer”, showed that the neutralizing buffer effectively neutralizes hydrogen peroxide disinfectant residual on a surface, maintaining the concentration of bacteria between the time of sampling and the time of assay. This provides validation of the methods for subsequent studies, in which sponge sticks will be used to sample surfaces exposed to hydrogen peroxide, the samples will be neutralized with neutralizing buffer, and a PBS-tween eluent, will be used to elute bacteria from sponge stick for spread plating.

The first experiment, which exposed the neutralizing buffer to MRSA was a critical preliminary step to ensure the buffer solution itself is not harmful to the test bacteria, meaning that it does not produce growth or die off of the bacteria during potential holding time between sampling and assay. The results found that there was not a significant change in concentration over time when MRSA and the neutralizing buffer were combined for 1 hour. This provides necessary information for not only further experiments within this study, but future studies using MRSA on surfaces.

Like the Osman et al. (2006) study, the neutralizing buffer was successful in eliminating the killing action of Sani-24. a variety of neutralizing broths have been developed, such as Letheen broth and Dey/Engley broth, which cover a variety of different disinfectants. For any
combination of neutralizer and bacteria, it is important to measure log reduction over time when bacteria are combined with the neutralizing buffer. When MRSA mixed with Sani-24 residual, the changes in concentration over time was not significant, providing further evidence that neutralizing buffer (monopotassium phosphate/sodium thiosulfate/aryl sulfonate complex) effectively neutralizes the bactericidal effects of Sani-24 over 1 hour. This data validates the choice of neutralizer for further tests examining Sani-24 efficacy.

Eluent is used as a liquid to aid the removal of bacteria or viruses during surface sampling (Julian, Tamayo, Leckie, & Boehm, 2011). Similar to the previous experiments, the eluent was important to test to ensure the PBS+0.01% Tween 80 solution did not kill the MRSA itself to ensure proper testing methods are used in the future. The results when testing MRSA with the eluent, Sani-24, and neutralizing buffer found no significant relationship between the log reduction and eluent solution. This data is important not only for future studies, but essential to evaluate the neutralizing buffer’s properties when the eluent is added. The results when testing MRSA with the eluent, Sani-24, and neutralizing buffer found no significant changes in the concentration of MRSA over 1 hour of exposure.

For any surface sampling method, it is important to understand the efficiency of recovery of organisms from the surface by the method. Here, swabbing of a surface contaminated with MRSA using a sponge stick soaked in neutralizing buffer, followed by elution with PBS/Tween, produced good recovery of MRSA. Keeratipibul et al. (2017), which examine the effect of different swabbing techniques, found cellulose sponge to be an effective method for recovery of S.aureus from surfaces, with 55.0% recovered from a stainless steel surface. The results with eluent and a rolling pin massage was found to be an effective method for recovery, with 63.78% of MRSA recovered from a stainless steel surface.
This showed that the proposed surface sampling methods produced acceptable recovery and were not harmful to the test bacteria. The neutralizer and eluent solutions did not affect the concentration of the bacteria of interest. The sponge stick recovery evaluated the use of a cellulose sponge stick. This study was important to pave the way for proper sampling and testing methods involving Sani-24. Moving forward, when future studies are conducted to examine the bactericidal efficacy of Sani-24 methods have already been tested and validated.

5.4 Challenges

While this study provided important data for future prolonged surface sampling studies, it did come with some challenges. Initially, the spreading technique proposed some issues due to lack of practice and perfecting the technique. This was seen a little bit within the experiment involving the neutralizing buffer, Sani-24, and eluent. In addition, the Baird-Parker plates were being read at the 24-hour mark, leaving the plates more difficult to read. The protocol was adjusted to read at the 24-hour and 48-hour mark to ensure the correct number of colonies were being recorded.

5.5 Conclusion

- The proposed surface sampling methods produced acceptable recovery and were not harmful to the test bacteria.
- The neutralizer and eluent solutions did not affect the concentration of the bacteria of interest.
- The sponge stick recovery evaluated the use of a cellulose sponge stick.
• Moving forward, when future studies are conducted to examine the bactericidal
efficacy of Sani-24 methods have already been tested and validated.
Works Cited


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the number of microorganisms. *Journal of Ayub Medical College Abbottabad, 31*(4 Sup), 646-650.


Stratton, H. Residential Swimming Pools and Spas.


6. TABLES

*Table 1. Recovery of MRSA from Sponge Stick*

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7. FIGURES

Figure 1. MRSA concentration ($\log_{10}(N_t)$) over 1 hour of exposure to neutralizing buffer

![MRSA Exposure to Neutralizing Buffer](image1)

Figure 2. MRSA and neutralizing buffer: Log Reduction ($N_t/N_0$) Vs. Time

![MRSA Exposure to Neutralizing Buffer](image2)
Figure 3. MRSA concentration ($\log_{10}(N_t)$) over 1 hour of exposure to neutralizing buffer and Sani-24 residual

Figure 4. MRSA, neutralizing buffer, and Sani-24 residual: Log Reduction ($N_t/N_0$) Vs. Time
Figure 5. MRSA concentration (over 1 hour of exposure to neutralizing buffer, Sani-24 residual, and eluent)

Figure 6. MRSA, neutralizing buffer, Sani-24 residual, and eluent: Log Reduction ($N_t/N_0$) Vs. Time

Figure 7. Recovery of MRSA from Sponge Sticks: Log concentration ($\log_{10}(N_t)$) vs. bag number
Recovery of MRSA from Sponge Sticks

![Bar chart showing log concentration of MRSA recovery from sponge sticks across different bags.](chart.png)