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Inoculation and Recovery of Test Organism Cocktail for Hospital Surface Disinfection by Sani-24 Wipes

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Inoculation and Recovery of Test Organism Cocktail for Hospital Surface Disinfection by Sani-24 Wipes

by

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Under the Direction of Lisa Casanova, PhD

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Abstract

Hospital-acquired infections (HAI) are a public health crisis that affect patients and hospital staff nationwide. A common bacterium that is linked to hospital-acquired infections is Methicillin-Resistant Staphylococcus Aureus (MRSA). In an effort to combat hospital-acquired infections and decrease bacteria on hospital surfaces, hospital staff has adopted disinfectant protocols which consist of the use of commercial hospital brand disinfectant wipes known as Sani-24. Sani-24 wipes claim to kill 99.9% of bacteria present on a surface after a 5-minute period. Sani-24 wipes also claim to continue to disinfect and inactivate bacteria on a surface a wipe has been previously used on. This experimental study aims to explore bacterial reduction with the use of Sani-24 wipes on hospital surfaces inoculated with MRSA. The results of this study display that there is not a consistent association of bacterial reduction between the six different time points. The results from this study may offer insight to healthcare administration and hospital staff on surface disinfection protocols to reduce bacteria contamination.

INDEX WORDS: Methicillin Resistant Staphylococcus Aureus (MRSA), Hospital Acquired Infections (HAI)

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Author's Statement Page

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Thesis Summary

Inoculation and Recovery of Test Organism Cocktail for Hospital Surface Disinfection by Sani-24 Wipes

Purpose: The purpose of this research was to measure bacterial reduction after using Sani-24 disinfectant wipes for the inactivation of *methicillin resistant staphylococcus aureus* and bacteriophage MS2 on the surface of durable hospital equipment.

Methods: Using an experimental study design, organisms were inoculated onto the surfaces as a cocktail. Organisms were recovered by swabbing with neutralizing buffer (sodium monopotassium, sodium thiosulfate, aryl sulfonate complex) which was then assayed to measure bacterial and bacteriophage growth. Every hour post-disinfection, a set of test and control surfaces were inoculated to simulate recontamination and sampled for organisms after the recommended 5-minute surface contact time. In this experiment, test and control surfaces were inoculated with organisms immediately after disinfection at time 0 and sampled at 5 minutes. In the next step of this experiment, test and control surfaces were inoculated for one-hour post-disinfection and sampled for 5 minutes. They were also inoculated for 2 hours post-disinfection and sampled for 5 minutes, and up until surface set was calculated for each post-disinfection time point.

Conclusions: The recovery of organisms exemplified that the Sani-24 disinfectant wipes did not eliminate all the bacteria present on a hospital surface.

CHAPTER 1

Introduction

Hospital-acquired infections have become a premiere issue in the field of healthcare. Hospital acquired infections are also known as the term hospital-associated infections (HAI). According to the Centers for Disease Control and Prevention, about 1 in 43 residents in a hospital setting obtains at least one infection (CDC,2022).A common hospital acquired infection is methicillin resistant staphylococcus aureus (MRSA). In healthcare facilities such as hospitals or nursing homes, MRSA can cause severe problems including bloodstream infections, pneumonia, surgical site infections, sepsis, and death" (CDC, 2019).

Hospital-acquired infections also raise issues because they could complicate a preexisting condition a patient might have prior to coming to the hospital to seek aid. Complications of preexisting conditions associated with hospital-acquired infections can vary from being treatable to being lethal to patients (Lobdell et al., 2011). The knowledge of the potential of health complications correlated with hospital-acquired infections has prompted hospitals nationally to adopt extensive disinfection protocols (Montero et al., 2022). Among the sanitization methods that hospital staff adheres to is the practice of wiping down surfaces that patients come into proximity with disinfectant wipes. Disinfectant wipes are used in hospital settings to decrease the number of microbes present on the surface. We are proposing the following research questions: (1) What is the relationship between bacterial and bacteriophage reduction and the Sani-24 disinfectant wipes? (2) What is the effect of time on the use of Sani-24 disinfectant wipe and and bacterial and bacteriophage reduction? 3) How does this differ comparing two hospital surfaces and which will result in the most viral and bacterial reduction? 4) Does the Sani-24 disinfectant wipe eliminate all bacteria present on a hospital surface?

CHAPTER 2

Review of Literature

2.1 Overview of Disinfectant Wipes

2.1.1 History of the Wet Wipe

In 1957 the very first wet wipe was created by an individual by the name of Arthur Julius in the United States of America (Albaad, 2022). The original wet wipe did not have the intention of becoming a disinfectant tool but served the purpose of a cosmetic material Albaad, 2022). The original wet wipe was a simple piece of fabric soaked in a water-based solution. A year after Arthur's invention, he trademarked the wet wipe as the Wet Nap. Wet-Naps' initial intention was to improve skin hygiene (Albaad, 2022).

2.1.2 Public Knowledge of the Wet Wipe

The original wet wipe developed by Arthur Julius was locally known but not public knowledge until the year 1960 Albaad, 2022). In 1960 the original wet wipe known as Wet-Naps was introduced to the public due to the platform provided by a restaurant show (Albaad, 2022). Wet-Naps were purchased by the owner of the fast-food restaurant chain, Kentucky Fried Chicken for the use of his customers (Albaad, 2022).

Following the restaurant show where the wet wipes were first introduced to the public Wet- Naps began to be referred to by different names (American Deli, 2023). Other names that the Wet Nap can be referred to are Baby Wipes or Moist Towelette.

2.1.3 The Transfiguration of the Wet Wipe

The original wet wipe was trademarked as a woven piece of fabric soaked in water, but since then it has come a long way. Wet-Naps began serving the purpose of improving skin hygiene but

became a multipurpose product when it began being sold to restaurant owners Albaad, 2022). During the year 1970, Wet-Naps graduated from being a woven piece of fabric soaked in water to a disposable piece of non-woven piece of fabric. The manufacturing of Wet-Naps ultimately changed in 1970 (Albaad, 2022).

2.1.4 Wet-Wipes Today

Wet Wipes today still serve the purpose of cosmetology purposes in addition to personal or household use (Hadley et al., 2023). Like its origin, the majority of wet wipes sold are manufactured in the United States of America. By 2019 wet wipes have become a lucrative market. In the United States, wet wipes have become a household staple. Wet wipes have become a multipurpose household item (Braian et al., 2020). Wipes can be used for people of all ages from infants to the elderly (Braian et al., 2020). The types of wipes that are appropriate per age group can vary because today, there are many different types, brands, and varieties of wet wipes (Hadley et al., 2023). The fact of the matter is there is a wet wipe that is a wet wiped for all types of people. Wet wipes have become a 14 billion market (Albaad, 2022).

The issue of hygiene is a matter that dates back as early as the 18th century. Poor hygiene has been associated with adverse health effects since the beginning of time (Hartmann, 2020). Wipes were first used as a tool to increase the cleanliness of the hands (Albaad, 2022). In 1847 Ignaz Semmelweis discovered that hands played a large role in the transmission of bacteria and a large role with infection. Hand hygiene was then highlighted as an important topic because the correlation between infection and dirty began to prove itself during early studies (Hartmann, 2020). The original wet wipe created by Arthur Julius was challenged due to the fact many scientists did

not feel as though the wipes did not decrease bacteria the way they felt that it should. Early hospital officials claimed that clean hands were the best defense against hospital acquired infections (Hartmann, 2020). Williams et al., 2020 study displayed the correlation between bacterial reduction and disinfectant wipes.

2.2 Efficacy of Disinfectant Wipes

(William et al., 2010) developed three-stage protocol to analyze the efficacy of disinfectant wipes in hospital settings. This study used the bacterium Methicillin Resistant Staphylococcus Aureus. MRSA was inoculated onto stainless steel materials and the wipes being tested immediately followed the inoculation. The wipes that were tested overall proved to be effective due to the fact that there was a significant bacterial removal revealed versus the wipes that were used as a control. The test wipes (William et al, 2010) are the recommended brand that hospitals should implement in their disinfectant protocols because of its bacterial reduction potential.

2.2.1 Disinfectant Wipes in Hospital Settings

(Moccia, et al., 2020) conducted a study discussing decontamination approaches to decrease the number of harmful microbes present in hospital settings. The spread of microbes in hospital settings has presented a concern (Moccia et al., 2020). It is difficult for hospital staff to make a continuous effort of wiping down high-touch surfaces due to the high amount of existing responsibility. The purpose of the (Moccia et al., 2020) study is to evaluate existing sanitization procedures involving disinfectant cloths. (Moccia et al., 2020) revealed that the use of disposable wet cloths provided many advantages. The usage of disinfectant cloths allowed for rapid sanitization within the rooms of patients. Overall, this study (Moccia et al., 2020) provides the

knowledge that the usage of disinfectant cloths provides less exposure to harmful microbes and reduces the risk of contamination.

2.3 Overview of Methicillin-Resistant Staphylococcus Aureus

2.3.1 History of Methicillin-Resistant Staphylococcus Aureus

Staphylococcus Aureus is a type of bacteria that is known to cause staph infections. Methicillin-Resistant Staphylococcus Aureus is also known as MRSA. MRSA is known to be difficult to treat due to its resistance to antibiotics (CDC, 2023). MRSA was first observed during the year 1960 (Harkins et al., 2017). Scientists did not know too much about the bacterium. This curiosity is what prompted several tests to determine the biological composition and effects of the bacterium. The first description of MRSA was published by Margaret Patricia Jevons. Jevons was employed at the Public Health Laboratory Service in Colindale, London (Cookson, 2011). MRSA was first introduced in North America during the 1970s (Cookson, 2011).

2.3.2 Symptoms of Methicillin-Resistant Staphylococcus Aureus

Methicillin Resistant Staphylococcus Aureus poses as a threat to patients hospitalized because the symptoms acquired from this bacterium can provide complications to preexisting conditions (Jencson et al, 2022). Most MRSA skin infections can appear as a bump or lump on the skin (CDC, 2019). MRSA can lead to the following in healthcare settings, bloodstream infections, pneumonia, or surgical site infections (CDC, 2019). Symptoms of MRSA are known to have the potential to be serious, but in most cases, MRSA infections are not usually life-threatening (WebMD, 2023).

Many people who are infected by MRSA mistake the appearance of their infection for spider bites (Jencson et al, 2022).

2.3.3 Transmission of MRSA

Methicillin-Resistant Staphylococcus Aureus is mainly spread through infected people encountering noninfected people (Williams et al, 2010). In addition to person-to-person contact, MRSA can also be transmitted via objects carrying the bacteria (Castro, 2021). MRSA can spread through cuts or even scrapes. Skin-to-skin contact is the most common way MRSA spreads to another person (Cookson, 2011). MRSA overall is diverse in the way one can come into contact with the bacteria (CDC, 2022). MRSA is not an airborne mode of transmission like another bacterium (Cookson, 2011).

2.3.4 Susceptible Populations of MRSA

MRSA is a common bacterium that is present in enclosed, populous spaces. MRSA outbreaks have been noted in military training camps, childcare centers, hospitals, and jails. Hospitals are common for MRSA infections (Castro, 2021). One of the most notable hospitals acquired infections is MRSA infections (Jencson et al, 2022). The mortality rate of hospital acquired MRSA infections stands at 29% (Nguyen, 2022).

2.3.5 Methicillin Resistance Staphylococcus Aureus Presence in Healthcare Facilities

Occupational places are known to house bacterium, healthcare facilities are no exception. The bacterium can travel throughout a healthcare facility via staff or improper disinfection handling of

a surface (Cookson, 2011). "Approximately 5% of patients in U.S. hospitals carry MRSA in their nose or on their skin" (CDC, 2022). Herwaldt 2004, suggests that improper handling is a leading cause of MRSA presence in healthcare settings.

2.3.6 Antibiotic Resistance of Methicillin-Resistant Staphylococcus Aureus

A large public issue is an antibiotic resistance. Antibiotics are used often in hospital and clinical settings to kill or decrease the number of bacteria present in a person's body (Williams et al, 2010). Some bacteria strains are so incredibly difficult to kill to the point where they will literally resist the effects of antibiotic treatment (Bachmann, 2012). MRSA is a bacterium that is resistant to antibiotics and is difficult to kill via antibiotic treatment. MRSA's resistance to antibiotics poses an issue to patients diagnosed with MRSA because of the difficulty of treatment (Williams et al, 2010). "Antibiotic resistance is becoming a pressing issue in modern healthcare, and we are in serious danger of entering a post-antibiotic era. Current tests for MRSA tend to be expensive and not very fast" (Bachmann, 2012).

2.4 Overview of Hospital Acquired Infections

2.4.1 Hospital-Acquired Infections in Hospital Settings

Hospital-acquired infections are infections that are acquired throughout a patient's duration in a hospital setting that was not previously present (Monegro et al., 2022). Hospital-acquired infections can also be known as the abbreviation HAI (Monegro et al., 2022). Another term for hospital acquired infections is healthcare acquired infections. Hospital acquired infections are classified as any infection that surfaces that was not present prior to receiving care. Hospital

acquired infections pose issues because they can complicate preexisting health conditions (Lobdell et al., 2022). MRSA is a bacterium that is known to be a common hospital acquired infection in hospital settings (Sutton & Steiner, 2016).

2.4. 2 Tests Used to Determine Hospital Acquired Infections

Infections can vary therefore the testing used to determine whether a specific infection is present can vary too. (Lobdell., et al 2012) reveals that a common term in the hospital setting that serves as a clinical definition for when tests implicate a hospital piece of equipment as the source of infection as CRSBI. CRSBIs are more specific to patients that use catheters. Another test revealed by Lobdell., et all to determine a hospital infection is SSI. In other cases, bacteria culture tests are tests that can be used to discover any type of harmful bacteria present in your body (MedlinePlus, 2023). Bacteria culture tests often utilize biomarkers like blood, urine, and skin. The types of samples that are needed depend on the location of the infection (MedlinePlus, 2023).

The University of Edinburgh conducted a study to detect the bacterium MRSA using small sensors. The test detecting MRSA was designed by the University of Edinburgh. Like most bacterial culture tests, The University of Edinburgh utilized skin biomarkers by swabbing wounds and sores (University of Edinburgh, 2012). The main purpose of this study was to display wounds and lesions and to detect Methicillin Resistant Staphylococcus Aureus. The swabs are then analyzed by the electrical sensors that can detect MRSA. According to the University of Edinburgh, a sensor test is an effective method to identify MRSA which can also be referred to as a superbug.

CHAPTER 3

Materials and Methods

We tested two types of surfaces in this project. The surfaces in this project were two types of durable hospital equipment- an IV pump and a standard adjustable plastic hospital bed rail. The size of the surfaces to be tested was standardized with templates placed on the surface. Six trials per experiment were done using six individual squares including - three control surfaces and three experimental test surfaces Each of the surfaces were inoculated with 50 microliters of

5.6 X 10^6 concentration of MRSA which was spread in a thin film over the surface using a wooden stick.

During this experiment, the controls and test trials were inoculated with MRSA or bacteriophage organisms immediately after disinfection at time 0 and then sampled with a neutralizing buffer sponge. After that, test and control surfaces were inoculated with MRSA one hour after disinfection with the Sani-24 disinfectant wipes and samples for 5 minutes. Lastly, the surfaces were once again inoculated with MRSA and MS2, but this time 2 hours post disinfection and then sampled at 5 minutes. The process of inoculation with MRSA post-disinfection continued until the last surface set was inoculated 6 hours post-disinfection. Log10 inactivation of organisms after 5-minute surface contact time was calculated for each post-disinfection time point.

3.1 Preparation of Inoculum

The MS2 and MRSA were defrosted in a water bath. Post-thawing of the stocks, the stocks were then centrifuged for 10,000 rpm for 3 minutes to pellet the bacteria.. The supernatant from the bacteria tubes was immediately drawn out and discarded. Then, 500 microliters of Phosphate Buffered Saline are then added to each of the MRSA tubes. Following the PBS addition, the tube was vortexed to resuspend the pellet. Once all the pellets in the tubes were resuspended with PBS, 100 microliters of fetal bovine serum were added to simulate the proteins found in human body fluids. All the tubes are then combined in a 15ml centrifuge tube.

3.2 Preparation of Overnight Host

The overnight host was composed of 50 ml of tryptic soy broth (TSB). The host bacterium, E. coli Famp (ATCC #700891) was added to 50ml of TSB in a sterile shaker flask after it was thawed out and then pipetted into the shaker. The sterile flask containing the *E. coli* Famp (ATCC #700891), and 50 ml of TSB is incubated at 37 degrees Celsius and 100 rpm for 24 hours on a shaker platform.

3.3 Preparation of the Exponential Phase for growth of MS2

The exponential phase host for MS2 was prepared on the day of the experiment prior to the execution of the experiment. For every 100 ml of TSB, one ml of the host made the day prior was added to the new additional shaker flask. The new additional shaker flask used for the exponential phase has a larger volume. After contents were added to the additional flask, the new flask was placed on the shaker and incubated at 37 degrees Celsius and 100 rpm for 2-4 hours until the bacteria entered the exponential growth phase for the MS-2 assay.

MRSA ASSAY

A dilution series using 5 ml tubes was created for the bacteria. Then, 100 μL of MRSA was added to 900 μL of tryptic soy broth and then serially diluted 7 more times. Post dilution of the MRSA, the dilutions were then plated on to Baird Parker petri dishes. Each dilution was plated in duplicate. The Baird Parker petri dishes were incubated at 37 degrees Celsius for 48 hours (about 2 days). After incubation, the colonies present on the Baird Parker petri dish were manually counted and then recorded.

MS2 Assay

25 ml of supernatant of the MS-2 virus was drawn off from the centrifuged sample and placed into a 50ml centrifuge tube. Then, 100 μL of the virus was added to 900 μL of TSB and serially diluted six more times. To assay the sample, 1ml of the exponential phase host and the serially diluted MS2 samples were added to the 10 mL of top agar. After the exponential phase was added 1 ml of each sample would then be pipetted into each tube of top agar and host. The mixture was poured into petri dishes. This was incubated at 37 degrees Celsius for 48 hours (about 2 days). Next, the incubated plates were examined for plaque forming units, counted, and recorded.

CHAPTER 4

Results

A total of 6 time points were examined during this experiment. This experiment examined the reduction of bacteria with the usage of Sani-24 Disinfectant Wipes on hospital surfaces over 6 hours. Figure 1 depicts the decrease in log10(Nt/N0) for MRSA for both the IV Pump and Bedrail surface. Figure 2 depicts the decrease in log 10(Nt/N0) for MS2 for both the IV Pump and Bedrail surface. The Y- axis displays the reduction in in log10 (Nt/N0), and the x-axis represents the time points used in this experiment (1 to 6 hours).

MRSA Reduction

Reductions were documented but with broad variability as shown in figure 1. Reduction ranged from 0.85 log 10 units to -2.95 log 10 units during successive time points for the IV Pump inoculated with MRSA. For instance, during the 2-hour time period we saw the greatest reduction (-2.95 log10 units), while the 3 hour time point saw the least decline of (–0.85 log10)units. There were no measurable reductions during the four-hour time period and a small reduction was noted during the 6-hour time period.

During the one-hour time point for the bedrail inoculated with MRSA there also was no measurable reduction in the number of bacteria present, with various degrees of reduction seen in successive time points for the bedrail inoculated with MRSA. For instance, the highest decrease was 2.49 log10 units and we observed this during the 2-hour time point. The lowest reduction was observed at the 3-hour time point at –1.19 log 10 units. During the four-hour time point there were diverse degrees of reduction with the highest drop being 1.47 log 10 units. We observed there was no measurable reduction at the 5-hour time point and a small reduction was noted during the 6hour time point, like the MRSA reduction results of the IV Pump.

MS2 Reduction

During the one-hour time point, there was no measurable reduction in the number of virus particles present with various degrees of reduction seen at successive time points for the IV pump and bedrail surfaces inoculated with the virus. The highest decrease was seen at 2.13 log 10 units during the 2–hour time point, while the lowest was viewed at the 5–hour time point at –1.04 log 10 units. There was no measurable reduction at the 5-hour time point and a small reduction was noted for the 6-hour time point. During the one hour time point there was no measurable reduction in the number of virus particle present with various degrees of reduction seen at successive time points for the bedrail inoculated with MS2. The highest decrease was seen during the 2 hour time point at 2.49 log10 units. The lowest decrease was viewed during the 3 hour time

point at -0.9 log10 units. At the 4 hour time point there were varying degrees of reduction with the highest drop being noted at 1.47 log10 units. There was no measurable reduction at the 5 and 6 hour time point.

Figure 1. MRSA (Log10 (Nt)) Reduction

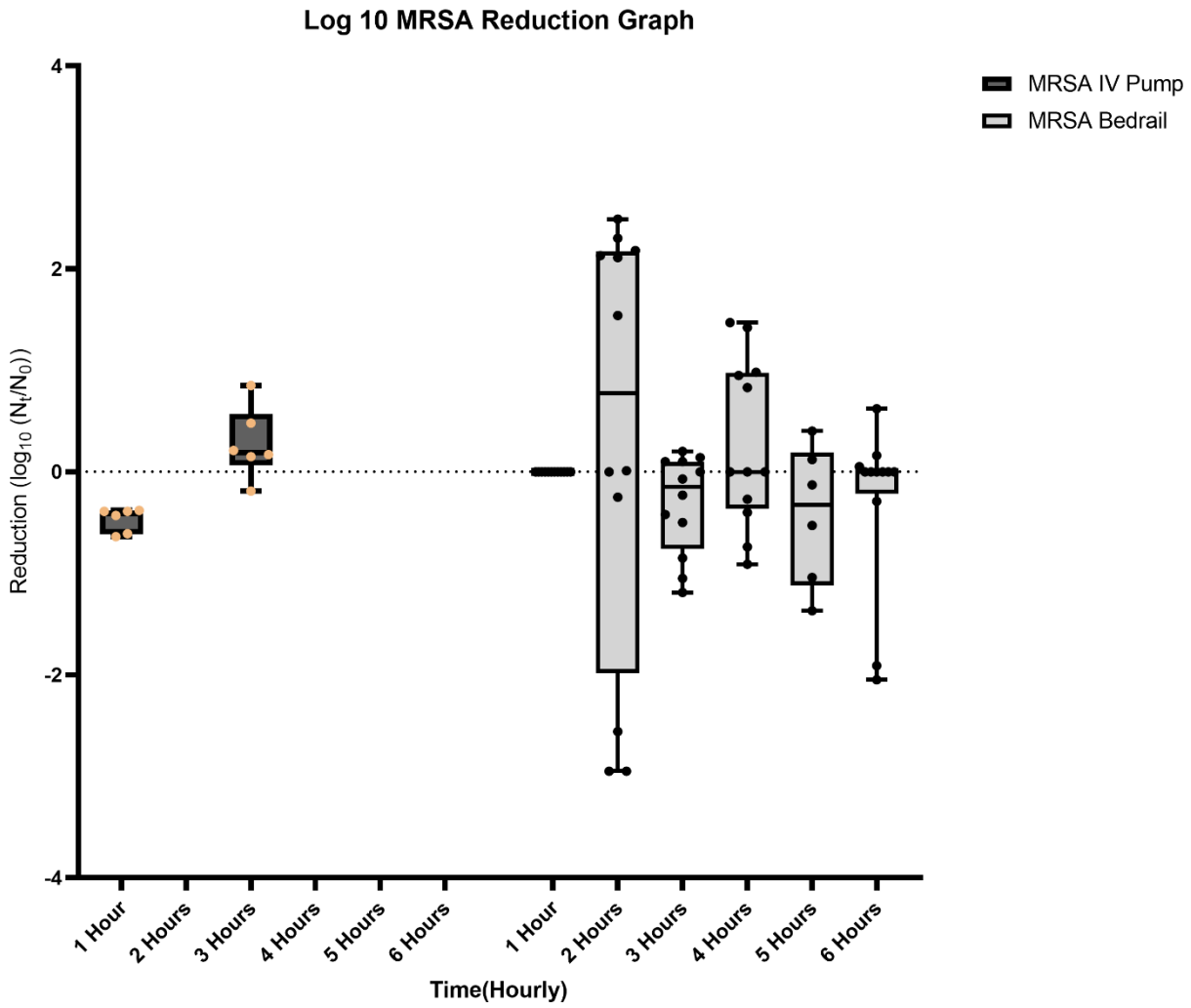
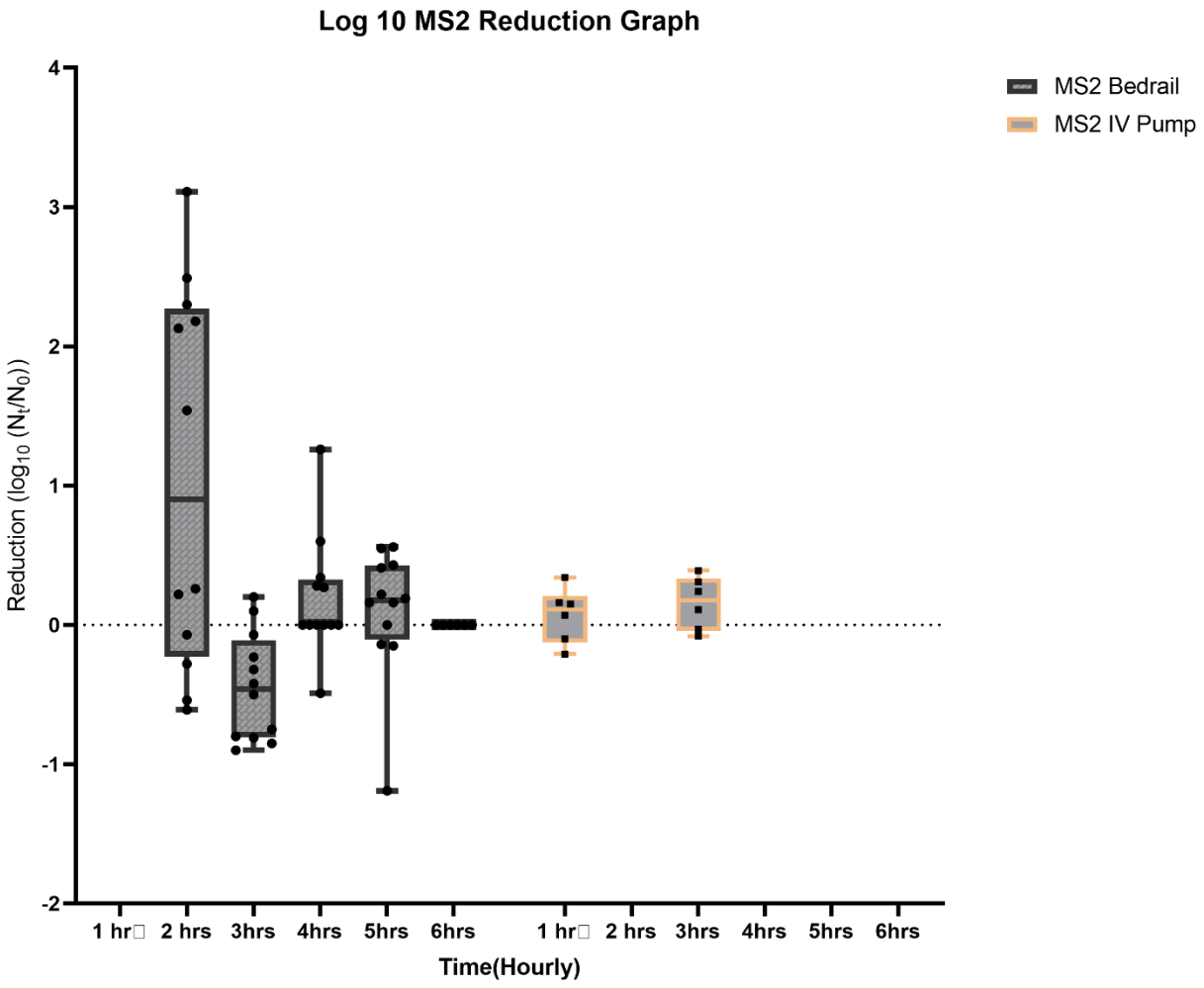


Figure 2. MS2 (Log10 (Nt)) Reduction



CHAPTER 5

Discussion and Conclusion

The results of this experiment displayed that Sani 24 wipes do not result in consistent reduction of the target bacterium over an interval of contact with sampling solutions for six hours. Sani 24 wipes are used in hospital settings to decrease the number of microbes on high touch surfaces. The results of this experiment provide evidence about how the microbes respond to Sani-24 wipes in hospital settings. This data is not only important for the public, but it is vital information for hospital organizations. Hospital organizations could use this data as they generate sanitization/disinfection practices for their facilities.

The results from the surfaces inoculated with MRSA and MS2 revealed there is bacterial reduction taking place after the disinfectant wipe is used. This overall provides validation for subsequent studies in which disinfectant wipes are used to decrease bacteria on durable hospital surfaces.

Several studies have been performed to test the bacterial reduction of MRSA using disinfection wipes. According to a study published in the American Journal of Infection Control, Cheng et al (2011), disinfectant wipes were effective in reducing MRSA contamination on hospital room surfaces. In comparison to Cheng et al (2011) the results from this experiment indicate that bacterial and viral reduction can be observed after the use of disinfectant wipes. In Cheng et al 2011 there was reduction of the bedrail inoculated with MRSA ranged from –0.70 log 10 units to

–1.65 log 10 units while in this study the bedrail inoculated with MRSA ranged from 2.49 log 10 units to 1.47 log 10 units. The main difference between Cheng et al 2011 and this experiment is that Cheng et al 2011 primarily focused on one durable hospital surface, patient bedrails, and this study explored two durable hospital surfaces, an IV Pump and a Bedrail. In addition, another difference that was noted during the evaluation of the Cheng et al study is that Another study that revealed similarity is Williams et al 2010. Like the Williams et al (2010) study, the sampling bacterial reduction was not consistent during time points. In this experiment there were time points that graphically had more reduction than other time points. This could be attributed to bacteria's nature to die off due to a variety of factors (Lewis, 2020).

The results from this experiment indicate that the bedrail inoculated with MRSA has a higher reduction in bacteria than the IV Pump, with the greatest reduction being visible during the two-hour time point. However, there were no measurable reductions in both the bedrail and the IV pump were observed during the 1-hour and 6-hour time periods. Similarly, the bedrail inoculated with MS2 had more reduced viral particles than the MS2 IV Pump during the 2-hour time point. We observed a pattern during the 1 hour and 6-hour time points by having no measurable reductions in both the bedrail and IV Pump for surfaces inoculated with both MRSA and MS2. Another pattern observed is that the two-hour time point has the greatest point of reduction for both organisms. Adding on, another result that surfaced during the duration of this experiment occurred during the time point one hour when more bacteria were present on the control surface post inoculation from when the experiment began. Bacteria attaching to surfaces has been a topic discussed for decades (Tuson & Weibel, 2013). Attachment to surfaces often stimulates bacterial growth (Tuson & Weibel, 2013).

Limitations

One limitation we noticed was that this study only looked at two durable hospital surfaces, so the bacterial and viral reduction on other hospital surfaces is unknown. Another limitation observed is that this study was conducted in a laboratory and may not reflect real-world conditions in hospitals or other healthcare facilities.

Conclusions

- **Moving forward, when future studies are conducted to examine bacterial and viral reduction on hospital surfaces using San-24, reduction results on IV Pumps and Bedrails inoculated with MS2 and MRSA have been tested and validated.**
- **The log reduction values for MRSA were consistently high throughout the 6-hour time interval, with the highest reduction seen at the 2-hour time point.**
- **The bedrail surface had more reduction values than the IV pump surface.**

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