Running head: MRSA RESISTANCE

Antibiotic and Disinfectant Resistance of Methicillin-Resistant *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that has developed an increasing resistance to antibiotic drugs. This bacterium is very prevalent in hospitals but is becoming more prevalent in community-based settings. The goals of this research are to test the antibiotic sensitivity of two strains of MRSA, discover the proper disinfectants to use in households and hospital settings, and develop and test antibiotic derivatives to determine the future of antibiotic use against this bacterium. Research indicated that each strain was resistant to β-lactam antibiotics as well as other antibiotics. Each strain tested was unique in its resistance against antibiotics, thus proving there is a need to evaluate the proper antibiotic treatment given to patients with MRSA infections. Disinfectants with a low or high pH are more effective than disinfectants with a neutral pH around 7. When testing antibiotic derivatives, this research indicated that compounds that are amphipathic and contain electron-withdrawing groups have the greatest toxicity against MRSA.

Antibiotic and Disinfectant Resistance of Methicillin-Resistant *Staphylococcus aureus*

Introduction

Methicillin-resistant *Staphylococcus aureus,* or MRSA, is a species belonging to the genus *Staphylococcus* and the family *Staphylococcacea*. It is a bacterium that is capable of surviving in the presence of antibiotics and inducing many illnesses in humans. *Staphylococcus aureus* became resistant to Penicillin and Methicillin shortly after these drugs became available for use as antibiotics (Appelbaum, 2007). Penicillin was introduced in 1940, and as early as 1942, *Staphylococcus aureus* was reported to be resistant to the drug. Shortly after Methicillin was introduced in 1961, *Staphylococcus aureus* was documented to be resistant to it as well, yielding what we call today, Methicillin-resistant *Staphylococcus aureus,* or MRSA. Reports indicate that MRSA is not only resistant to Penicillin and Methicillin but also to the whole family of antibiotics known as β-lactams to which Penicillin and Methicillin belong. Various strains of MRSA have also been identified with resistance against glycopeptide antibiotics, such as Vancomycin (Garau, Bouza, Chastre, Gudiol, & Harbarth, 2009).

MRSA is mostly found in hospital environments, but there have been increasing infections outside the hospital in the community (Klevins, et al 2007). This increasing trend has generated concern because MRSA has the potential to be lethal. In August 2004, a study found that the main cause for skin infections presented in the emergency department was Methicillin-resistant *Staphylococcus aureus* (Moran, Krishnadasan, Gorwitz, Fosheim, McDougal, Carey, & Talan, 2006). There are two main strains of MRSA and each has different characteristics than the other. The first is called hospital-acquired MRSA, or HA-MRSA, and the second is called community-acquired MRSA, or

CA-MRSA. HA-MRSA infections, also known as nosocomial infections, and CA-MRSA infections are similar in that both are resistant to all β-lactam antibiotics. Nosocomial infections tend to be more resistant to antibiotics than CA-MRSA infections. CA-MRSA is more likely to be susceptible to ciprofloxacin, clindamycin, erythromycin, and gentamicin (Appelbaum, 2007). It was also found that CA-MRSA is more likely to be susceptible to trimethoprim-sulfamethoxazole, clindamycin, and fluoroquinolone (LaPlante, Rybak, Amjad, & Kaatz, 2007).

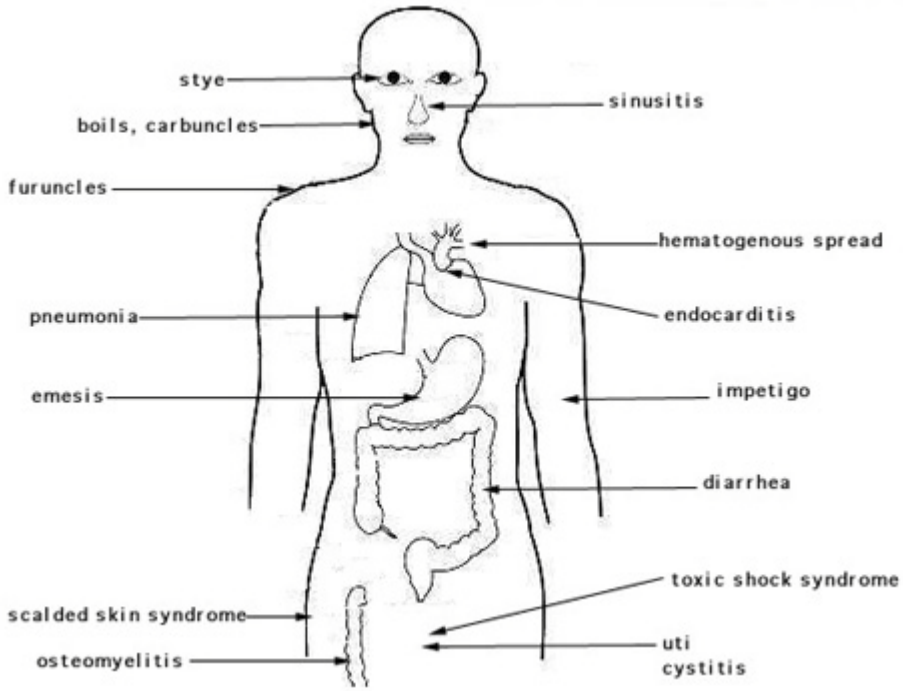

Figure 1. Parts of the body and illnesses caused by a MRSA infection. MRSA has the ability of infecting multiple parts of the body, thus producing a variety of illnesses (Image from Todar, 2008).

The reason for the differences of these two groups of strains is genetic. CA-MRSA infections typically have less resistance against antibiotics, contain a different subtype of staphylococcal cassette chromosome (SCC*mec*) IV, and carry a gene known as Panton-Valentine leukocidin (PVL) (Klevens, Morrison, Nadle, Petit, Gershman, & Ray, 2007, and Appelbaum, 2007). It is speculated that CA-MRSA infections may be more virulent than HA-MRSA infections due to the PVL gene. The PVL gene is a gene that creates Panton-Valentine Leukocidin cytotoxin which is responsible for some skin lesions as well as necrotizing pneumonia.

HA-MRSA and CA-MRSA infections can cause a multitude of illnesses depending on where the infection is located, as seen in Figure 1. The infections can cause anything from a small rash to death. Mostly found in children ages 2 to 5 years old, impetigo is a skin rash that is highly contagious and often caused by *Staphylococcus aureus* (Cole & Gazewood, 2007). This skin rash is simply a topical infection of the epidermis, the site of the body's first innate immune defenses. Bullous impetigo, which is characterized by fluid filled blisters compared to simple rashes on the skin in other cases of impetigo, is common with *Staphylococcus aureus.*

In the hospital, MRSA is found to grow more readily as biofilms on the catheters of dialysis patients, which could lead to urinary tract infections. The bacteria growing in biofilms on medical devices was found to be much more resistant to antibiotic and antimicrobial treatment than bacteria that are free living (planktonic bacteria). Specifically, bacteria living in a biofilm are about 1,000 to 1,500 times more resistant than planktonic bacteria (Wu, Kusuma, Mond, & Kokai-Kun, 2003). This resistance is because the bacteria have an increased interaction with each other. Devices that are

implanted into individuals such as pace-makers and shunts are also subject to biofilm formation which can cause infections (Saginur, Denis, Ferris, Aaron, Chan, Lee, & Ramotar, 2006). MRSA biofilms on pace-makers, prosthetic heart valves, and shunts can cause endocarditis, which is inflammation of the inside lining of the heart chambers and heart valves. Because of the difficulty in eliminating a biofilm on a medical device in a patient, a contaminated device that causes an infection must be removed or replaced. Although the risk of infection from medical devices and implants is low (between 1% and 7%), infections that occur are serious. Morbidity and mortality can follow, as well as causing an increased time in the hospital, more surgeries to replace the devices, and additional costs due to the extra health care involved.

Another condition caused by MRSA is osteomyelitis, which is an infection of the bone or bone marrow (King & Johnson, 2008). This condition can produce different symptoms depending on which bones are infected. For example, vertebral osteomyelitis is the infection of the vertebral column, and can result in neurological symptoms, fever, and edema. Osteomyelitis can be caused by direct inoculation of bacteria through surgery or an infection caused by bacteria flowing in the blood, also called bacteremia. Most children infected with osteomyelitis were found to have strains of MRSA with the Panton-Valentine Leukocidin (PVL) gene (Bocchini, Hulten, Mason, Gonzalez, Hammerman, & Kaplan, 2006). The strains with the PVL gene were shown to cause greater illness through increased systemic inflammation.

MRSA is capable of escaping the body's immune defenses in a variety of ways. MRSA releases exotoxins such as α-toxin and β-toxin that are able to lyse cell membranes (Todar, 2008). This bacterium also has the ability to latch onto the antibody,

IgG, through its surface protein, protein A. By binding to IgG in a different fashion than regular antigens, it decreases opsonization and thus phagocytic activity. Normally, IgG increases phagocytic activity by marking pathogens in the body to be ingested. MRSA can cause toxic shock by releasing superantigens (Ferry, Thomas, Genestier, Bes, Lina, Vandenesch, & Etienne, 2005). The superantigens are MRSA enterotoxins and toxic shock toxins that bind more strongly to parts of T-helper cells, thus causing an abnormal release of cytokines into the body. This influx of cytokines causes a more systemic inflammatory response, ultimately resulting in septic shock or toxic shock.

In community settings, MRSA is spread in areas where people live in close quarters and interact with each other often such as prisons, daycare centers, and schools. Skin to skin contact can cause MRSA to spread. *Staphylococcus aureus* exists as normal flora in the anterior nares, or the external portion of the nose, of all humans (Nicolle, 2006). In hospital settings, MRSA is present due to surviving on biofilms and being present as normal flora on the hands of hospital employees (Cimiotti, Wu, Della-Latta, Nesin, & Larson, 2004). Since *Staphylococcus aureus* exists as normal flora in humans, the bacteria cannot simply be eliminated from surgery rooms, hospitals, or even in the community. Careful procedures must be followed in order to avoid contamination of wound and surgery sites when in the hospital (Humphreys, Grundmann, Skov, Lucet, & Cauda, 2009).

Hospital-acquired and community-acquired MRSA strains are important to study in order to prevent illness rather than to just treat it. For this reason, this study examines the resistance of Methicillin-resistant *Staphylococcus aureus* against disinfectants, antimicrobial hand soaps, and a variety of antibiotics. The effectiveness of disinfectants

allows individuals to decide which cleaning products to use against MRSA. The disinfectants that are tested in this study range from household cleaners to cleaners used in surgical operating rooms. Antibiotic sensitivity tests aid in choosing the most effective antibiotic for recovery.

In addition to testing various disinfectants and known antibiotics, antibiotics that are not standard are being tested against these strains of MRSA. Imidazole, shown in Figure 2, Triclosan, shown in Figure 3, and their derivatives were researched. By combining the ideas and results of this research, more derivatives of Imidazole were created. The goal is to create compounds that are effective against MRSA.

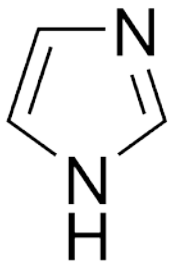

Figure 2. The molecular structure of Imidazole. Imidazole is used as a base compound in this research. Benzene rings as well as substitutions are made in order to increase its bacterial toxicity.

In a study using Imidazole, it was found that the presence of electron withdrawing groups was essential for antimicrobial activity (Sharma, Narasimhan, Kumar, Judge, Narang, Clercq, & Balzarini, 2008). This study used Imidazole as the base compound and attached two benzene rings to the Imidazole. The main base of the compound that produced positive results had two phenyl rings instead of one. This compound was called

(substituted phenyl)-[2-(substituted phenyl)-imidazole-1-yl]-methanone. One phenyl ring also had different substitutions to it while the other phenyl ring had only one substitution that changed. Antibacterial activity was highest when chlorine was placed in either the R1 or the R3 position on the phenyl ring and when carboxylic acid was placed in the R1 of the substituted phenyl ring.

Another compound that was studied is Triclosan, also known as 5-chloro-2-(2, 4-dichloropheoxy) phenol. Triclosan is an antimicrobial agent used in many households in mouthwashes, toothpastes, and soaps (Suller & Russell, 2000). It is also found in many plastics, fabrics, and kitchenware (McMurry, Oethinger, & Levy, 1998). Triclosan is very important because it is effective against *Staphylococcus aureus*, a broad range of both gram-positive and gram-negative bacteria and fungi (Stewart, Parikh, Xiao, Tonge, & Kisker, 1999).

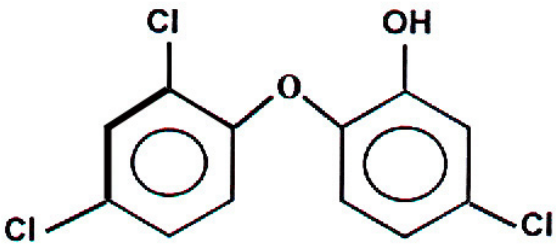

Figure 3. The molecular structure of Triclosan. Triclosan is an antimicrobial agent found in many commonly known hygiene products and is also found to be effective against Methicillin-Resistant *Staphylococcus aureus*.

Triclosan targets an enzyme that enables fatty acid synthesis in bacteria. The enzyme is known as enoyl-acyl carrier protein reductase, or ENR (Stewart et al., 1999).

By inhibiting this enzyme, fatty acid synthesis is disrupted, thus the membrane bilayers of the bacteria are not created. ENR catalyzes the NADH-dependent reduction of fatty acids bound to the acyl-carrier protein. More specifically, Triclosan targets the *fabI* gene. This gene codes for ENR (McMurry, Oethinger, Levy, 1998). This was determined through mutations of the gene and then comparing bacterial lipid synthesis of the mutated genes and wild-type genes (Stewart et al., 1999).

The effectiveness of Triclosan is brought about by its amphipathic characteristic. Surfactant products were found to have an increased toxicity to bacteria than products that were nonpolar (Green, Tocoli, Lee, Nihei, & Kubo, I. 2007). In a study, a side chain of carbon atoms was lengthened to test the toxicity of the compounds. Compounds with shorter side chains of carbons, and thus more polar, were found to be more toxic to bacteria. Researchers found that long carbon chains decreased the toxicity of the compound against bacteria, and that amphipathic molecules increased toxicity of the compound. Another study showed that electron withdrawing groups such as Cl^- increased toxicity as well (Sharma, et al, 2008). Another study showed that adding chlorine to the already chlorinated Triclosan increased its effectiveness. When comparing and combining these data, an Imidazole derivative that is effective against MRSA can be created. Since research indicated that chlorine and a phenyl group play important roles in toxicity, Imidazole can be modified in order to increase bacterial toxicity.

This study is divided into four areas of interest. The first area includes the isolation of this bacterium. The second area of interest is antibiotic sensitivity testing in which the effectiveness of several antibiotics employed by physicians are tested. The

third area involves testing MRSA against various disinfectants as well as antimicrobial hand soap. The final area of interest is testing derivatives of Imidazole to create a drug that is effective against MRSA.

Methods

Isolation of MRSA

The first step in this research is isolating Methicillin-resistant *Staphylococcus aureus* from the environment. The bacterium was acquired from Butner Federal Medical Center at the Butner Federal Prison. Three patients with known MRSA infections were swabbed with sterile swabs in order to obtain MRSA samples. The swabs were then collected in agar slants and sealed to prevent contamination of the environment. These agar slants were placed in biohazard bags to further ensure the prevention of contamination. The bags were transported to Liberty University and incubated at 37° Celsius to allow for growth. The second strain of bacteria was obtained by swabbing an infection from an individual's foot that was suspected of being caused by MRSA.

After a few days of growth, the bacteria were placed on mannitol-salt agar (MSA) plates in order to initially select for Staphylococcus bacteria. Mannitol-salt agar plates contain a salt concentration of 7.5 percent. Most bacteria are not able to survive in such a high salt concentration, but *Staphylococcus aureus* is able to survive in up to 15 percent salt concentrations (Todar, 2008). *Staphylococcus aureus* is mannitol-salt positive. Inoculation of MSA plates allowed for the initial isolation by observing which bacteria used the nutrients from the environment. This nutrient use was indicated by the plate's changing of colors from a red pigment to yellow around the bacteria. The color change indicates a positive pH change and the bacteria is labeled as mannitol-salt positive. Since

MRSA is known to be resistant to the antibiotic, Oxycillin, MSA plates that contained Oxycillin in the agar were made to isolate the bacteria further. Bacteria that grew on the agar and were mannitol-salt positive were used in additional tests. The bacteria that met the initial requirement of being mannitol-salt positive and Oxycillin-resistant were then observed for proper pigmentation. *Staphylococcus aureus* has been classified as having a yellow pigmentation (Todar, 2008). *Staphylococcus aureus* is also characterized as gram-positive and its shape is that of small, spherical clusters, as seen in Figure 4. The bacteria in this study were subjected to gram staining and observation. Also, trypticase soy agar (TSA) and blood agar plates were created. TSA plates simply allowed for growth of the bacteria, while blood agar plates tested whether the bacteria were hemolytic.

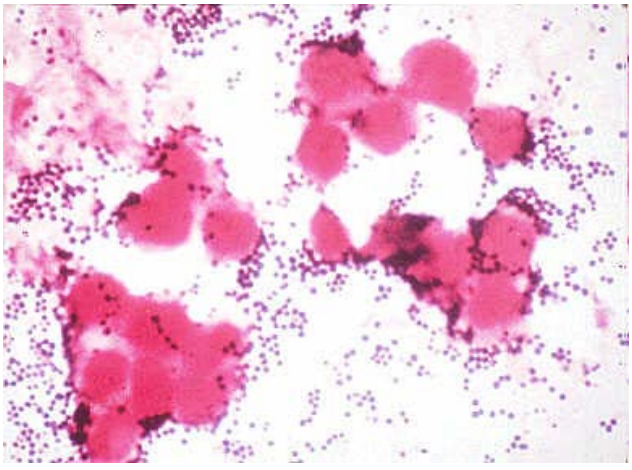

Figure 4. A picture of *Staphylococcus aureus* under the microscope after gram-staining. After gram-staining *Staphylococcus aureus* on a slide, it is noticeable that the bacterium is gram-positive by its purple appearance, and it is also identifiable as cocci by its spherical form (Image from Todar, 2008).

Catalase, oxidase and coagulase tests were performed. Catalase is an enzyme found in *Staphylococcus aureus* that enables it to convert hydrogen peroxide into water and carbon dioxide. The presence of catalase activity was tested by placing hydrogen peroxide over the bacteria and observing if bubbles were formed. The oxidase test tests for the presence of cytochrome C oxidase. In this test *N,N*-Dimethyl-*p*-phenylenediamine is used as a reagent on a paper disk. Bacteria are placed on paper disks for observation. If the bacteria changes from colorless to a dark color, the oxidase test is positive. If the bacterium on the disk does not change color, the oxidase test is negative. Since *Staphylococcus aureus* is known to produce the enzyme coagulase, a coagulase test was performed.

Furthermore, because *Staphylococcus aureus* is characterized as a facultative anaerobe, the bacteria were placed in an agar deep to test whether it was an anaerobe, aerobe, or facultative anaerobe. Each test was done at least twice in order to ensure accuracy and proper identification of the bacteria.

Antibiotic Sensitivity Tests

Antibiotic sensitivity tests for the two MRSA strains were performed by inoculating trypticase soy agar, or TSA, plates with TSA broth from each MRSA strain and placing antibiotic discs onto the plates. The broths were created in test tubes containing trypticase soy broth and bacteria from existing TSA plates. The broths were incubated at 37° Celsius for one week before being used for the antibiotic sensitivity tests. When the broths were ready for the antibiotic sensitivity tests, the absorbances of the broths were measured to ensure that the broth mixture did not have too high of a concentration of bacteria for the tests.

Once the absorbances of the broths were measured, 0.2 milliliters of the broth was spread over the plate in order to allow for uniform growth of the bacteria. Each TSA plate was split into four sections in order to avoid waste of agar and plates. The plates were incubated at 37° Celsius for one week before being examined. The antibiotic sensitivity tests were done twice for the MRSA strain extracted from the individual's foot, which will be called MRSA strain 1, and three times for the MRSA strain acquired from the prison hospital, which will be called MRSA strain 2, in order to have better accuracy. In the results, the average zone of inhibition of the trials was used. The zone of inhibition indicated the effectiveness of the product being tested. It was measured in millimeters from the middle of the disk to the outside edges of bacterial growth. The diameter of the disks is 8 millimeters. The MRSA were considered susceptible to any antibiotics with a zone of inhibition greater than 15 millimeters. The antibiotics tested were Doxycyline, Erythromycin, Vancomycin, Bacitracin, Oxycillin, Kanamycin, Steptomycin, Isonazid, Tetracycline, Ciprofoxacin, Ampicillin, Cepthlothin, Trimethoprim-Sulfamethoxazode, and Penicillin. The results to these tests can be found in Table 4.

Disinfectant Sensitivity Tests

Disinfectants and hand cleaning antimicrobial agents were tested in order to observe which products are the most effective in eliminating MRSA. The products tested were obtained from households, Butner Federal Medical Center, and Liberty University.

The disinfectants and solutions used in the experiment include The Works Toilet Bowl Cleaner, Kaboom Shower, Tub, and Toilet Bowl Cleaner, Hibiclens Clorohexidine Gluconate solution, Lysol 4 in 1, Rejuvnal HBV, Povidone, which is an iodine scrub,

Tilex Mold and Mildew, 70% ethanol, and hydrogen peroxide. The disinfectants were diluted into five different solutions including 100%, 50%, 25%, 12.5%, and 6.25%.

Table 1. *Active ingredients of disinfectants. The active ingredients of the disinfectants determine the effectiveness of the disinfectant and its dilutions.*

Active Ingredients of Disinfectants	
Disinfectant	**Active Ingredients**
Povidone	Povidone-iodine USP 7.5%
Clorox	Sodium hypochlorite
Hibiclens	Clorohexidine gluconate solution 4.0% w/v
Kaboom	Sodium hypochlorite, ammonia, lye, sulfuric acid
Grease Lightning	Sodium hydroxide, 2-butoxyethanol
The Works Toilet Bowl Cleaner	Hydrogen chloride, 20%
Lysol 4 in 1	Benzalkonium chloride
Rejuvnal	Didecyl dimethyl ammonium chloride, 2.54%, and n-Alkyl dimethyl benzyl ammonium chloride, 1.69%
Tilex Mold and Mildew	Benzalkonium cloride

The active ingredients of the disinfectants are listed in Table 1. Active ingredients are common in multiple cleaners. These include sodium hypoclorite in both Clorox and Kaboom and benzalkonium cloride in Lysol 4 in 1 and Tilex. The disinfectants with similar active ingredients should work about the same, with the exception that the concentration of the active ingredient may increase or decrease the effectiveness of the antibacterial properties of the disinfectant. The pH of the disinfectants were also tested and determined by using pH paper. The color on the pH

paper indicated the pH of the disinfectant or solution. The pH of each disinfectant and solution is listed in Table 2.

Table 2. *pH of the disinfectants and solutions. The pH of the disinfectants and solutions was determined by using pH paper.*

pH of Disinfectants	
Disinfectant	**pH**
Povidone	4.5
Clorox	10
Hibiclens	9.5
Kaboom	2
Grease Lightning	7.5
The Works Toilet Bowl Cleaner	0
Lysol 4 in 1	8
Rejuvnal	5
Tilex Mold and Mildew	10
Hydrogen Peroxide	4
70% Ethanol	5

The disinfectants were diluted through serial dilutions. Two milliliters of 100% solution were placed into a tube with two milliliters of deionized water and mixed. Two milliliters of that solution were then placed in another test tube containing two milliliters of deionized water and mixed. This process was repeated until the last test tube contained a 6.25% solution. Two milliliters of that solution were discarded due to keeping with a constant volume of two milliliters for each test tube. Disks of filter paper with a diameter of about 8 millimeters were placed in the tubes to soak for a few minutes before placing a disk onto an inoculated TSA plate to test the resistance of the bacteria.

In order to test the bacteria's resistance against the disinfectants and solutions, the bacteria were taken from a previously inoculated TSA plate to create a TSA broth. The broths were allowed to grow for 48 hours in an incubator at 37° Celsius. When taken out of the incubator, 0.2 milliliters of broth was used to inoculate each TSA plate for testing. Before inoculation of the plates, the absorbances of the broths were tested. The broth was spread over the plate in order to allow for uniform growth of the bacteria. Each TSA plate was split into four sections. They were labeled according to which disinfectant, solution, and their respective dilutions to be tested in the quadrants. 48 hours later, the bacterial growth was observed. Three trials of the disinfectant sensitivity tests were performed for both strains of MRSA. Tables 5a, 5b, and 5c list the effectiveness of the disinfectants, the solutions, and their dilutions in the trials. The zone of inhibition indicated the effectiveness of the product being tested. It was measured in millimeters from the middle of the disk to the outside edges of bacterial growth.

Antibiotic Derivative Sensitivity Tests

In preparation for testing the Imidazole derivatives against Methicillin-resistant *Staphylococcus aureus*, the drug solvents were tested before the derivatives were tested. This was to ensure that none of the solvents distorted the results from the Imidazole derivatives. The solvents that were tested were dimethyl sulfoxide (DMSO) and acetone. Table 6 lists the solvent dilutions that may be used, their dilutions ranging from 100% to 5% solution, and their respective zones of inhibition. The solvent testing was performed by using 0.2 milliliters of MRSA broth to inoculate a TSA plate. This was to ensure uniform growth so that results were not distorted in any way. Filter disks were soaked in

each dilution and then placed on an inoculated TSA plate. The plates were observed 48 hours after inoculation.

The compounds tested were 2-(3-chlorophenyl)-imidazole, 2-(2-chlorophenyl)-imidazole, 2-(4-chlorophenyl)-imidazole, 2-(4-phenyl)-imidazole, and 2-(4-methyletherphenyl)-imidazole. These compounds were created in the organic chemistry lab at Liberty University by Caitlin Hubbard. After discovering the best combination of solvents, the Imidazole derivatives were dissolved using a mixture of 5% DMSO and 10% acetone and then tested. A range of concentrations of the compounds were tested in which 0.5, 0.1, 0.01, and 0.001 mg/ml solutions of the compounds were created. The sensitivity tests for each compound were performed in the same manner as the other sensitivity tests. A TSA plate was inoculated with 0.2 milliliters of broth and a filter disk soaked with the liquid compound was placed on a TSA plate. The TSA plates were split into four quadrants representing varying concentrations of the compound being tested. These quadrants were 0.5, 0.1, 0.01, and 0.001 mg/ml solutions. The plates were incubated at 37°Celsius for three days and then observed. The zones of inhibition were measured in millimeters from the middle of the disk to the outside edge of inhibition where bacteria began to grow. The results can be found in Table 7.

Results and Discussion

Isolation of MRSA

After gram-staining, the bacteria in the study were found to be gram-positive and existing as small spherical clusters, which is consistent with *Staphyloccocus aureus*. The bacteria on the blood agar plates were hemolytic. The bacteria were also catalase positive, which is indicative of *Staphylococcus aureus*. The bacteria in this study did not

change color on the disk, indicating they are oxidase negative. Because *Staphylococcus aureus* doesn't exhibit oxidase activity, this is further evidence that these bacteria are *Staphylococcus aureus*. Since *Staphylococcus aureus* is known to produce the enzyme coagulase, a coagulase test was performed, and the bacteria in this study tested positive. Also, when the bacteria were placed in deeps, they grew down and throughout the agar deeps as well as at the surface of the agar, proving they are each facultative anaerobes. It is labeled as a facultative anaerobe because it has the ability to survive in environments with oxygen and in environments without oxygen. Table 3 lists the tests that were performed in order to verify the bacteria were MRSA. Each test was performed at least twice for each strain of MRSA.

Table 3. *The strains of bacteria were first isolated and subjected through a series of tests to ensure that they were indeed Methicillin-resistant Staphylococcus aureus.*

Isolation Tests	
Shape	Small, spherical clusters
Color	Yellow
Gram-Stain	Positive
Catalase Test	Positive
Oxidase Test	Negative
Coagulase Test	Positive
Mannitol Salt Agar (MSA)	Positive
Hemolytic	Positive
Deep	Facultative Anaerobe
Oxycillin	Resistant
Penicillin	Resistant

Each test result is characteristic of *Staphylococcus aureus*. Any bacteria that were not characteristic of MRSA were discarded. MRSA was separated from normal *Staphylococcus aureus* through the use of the mannitol-salt agar plates with Oxycillin. Normal *Staphylococcus aureus* is susceptible to Oxycillin, while it has been found that MRSA is resistant to Oxycillin. This is because Oxycillin belongs to the β-lactam family of antibiotics, which MRSA is resistant. Two strains of bacteria were isolated from these tests which were then grown on TSA plates and used in the antibiotic sensitivity, disinfectant sensitivity, and antibiotic-derivative sensitivity tests.

Antibiotic Sensitivity Tests

All tests were positive to be Methicillin-resistant *Staphylococcus aureus.* Before the antibiotic sensitivity tests began, the absorbance of each MRSA strain was measured. MRSA strain 1 was measured at 0.95 absorbance while MRSA strain 2 measured at 0.4 absorbance. In the antibiotic sensitivity tests, it was found that the strains of bacteria were resistant to Ampicillin and Penicillin, which belong to the β-lactam antibiotic family. Their resistance to β-lactam antibiotics further confirms that these strains of *Staphylococcus aureus* are MRSA strains.

In addition to each strain being resistant to Oxycillin, Ampicillin, and Penicillin, each strain was also resistant to Sulfamethoxazole-Trimethoprim, Isonazid, and Bacitracin. The results, which can be found in Table 4, indicate that both strains are susceptible to Vancomycin, Doxycycline, Streptomycin, Kanamycin, and Tetracycline. This means that if a patient is infected with these particular strains of MRSA, physicians could use these antibiotics to eliminate the bacteria. Choosing an appropriate antibiotic is important because all bacterial strains are different, as indicated by the data. Some

antibiotics inhibit MRSA growth while others do not. Ciprofloxacin and Erythromycin are examples in that each antibiotic inhibited the growth of one strain of MRSA, but not the other.

Table 4. *Antibiotic sensitivity tests. Various antibiotics were tested against Methicillin-resistant Staphylococcus aureus.*

Antibiotic Sensitivity Tests		
	Zone of Inhibition (mm)	
Antibiotic	**MRSA Strain 1**	**MRSA Strain 2**
Doxycycline	42	41.3
Ampicillin	0	0
Ciprofloxacin	21	0
Erythromycin	0	25.3
Vancomycin	24	21.3
Streptomycin	40	36
Sulfamethoxazole Trimethoprim	0	0
Kanamycin	32	27.3
Oxycillin	0	0
Penicillin	0	0
Tetracycline	34	34.7
Isonazid	0	0
Bacitracin	0	0

Examining this data can lead to other hypotheses. In a study, resistance to Erythromycin was found to expedite the ease of resistance to Clindamycin due to inducibility (Siberry, Tekle, Carroll, & Dick, 2003). MRSA isolates that were originally Erythromycin resistant, but Clindamycin susceptible, were discovered to be resistant

against Clindamycin when subjected to antibiotic treatment with Clindamycin. To hypothesize that the strain from MRSA strain 2 is susceptible to the antibiotic Clindamycin would be reasonable due to its susceptibility to Erythromycin. Likewise, MRSA strain 1 could be resistant to Clindamycin due to its resistance to Erythromycin.

Disinfectant Sensitivity Tests

MRSA is prevalent in both the healthcare and community settings, so it is important to exercise proper cleaning and sterilization techniques to decrease the chances of infection. The reason for diluting the disinfectants and solutions was to observe the effectiveness against the bacteria even in small quantities of active ingredients. Often disinfectants are used with water or simply diluted while cleaning. The effectiveness of the diluted concentrations indicates whether this is a safe practice or not. The reasoning behind testing hydrogen peroxide was to test a possible means of cleaning and getting rid of MRSA in the wound when infected with MRSA.

Before the disinfectant sensitivity tests began, the absorbances of the broths were measured. For all six trials of the tests, the broths of each strain had absorbances of approximately 0.7. Through antibiotic testing in recent research, it was found that amphipathic and electron-withdrawing compounds had a much higher rate of bacterial toxicity than non-polar and regular compounds (Sharma et al., 2008). This toxicity may also play a large role in the effectiveness of disinfectants against bacteria. Many of the disinfectants were listed as highly basic or highly acidic solutions because of their active ingredients. This could be indicative that ionization of parts of bacteria could disrupt structures and/or functions. Examples of compounds that were known to work against bacteria were compounds that included chlorine, carboxylic acid, or phenyl substituents.

Amphipathic solutions should be more effective against bacteria because they are universal solvents. *Like dissolves like* is a common phrase used in chemistry that applies to this situation. Being amphipathic, the compound has the ability to dissolve, bind, and/or disrupt both polar and nonpolar parts of an organism.

Table 5a. *Disinfectant sensitivity tests. The resistance of MRSA was tested against disinfectants. The zones on inhibition indicate the strength of each disinfectant against MRSA.*

Disinfectant Sensitivity Tests			
		Zone of Inhibition (mm)	
Disinfectant	**Dilution (%)**	**MRSA Strain 1**	**MRSA Strain 2**
Povidone	100	20.7	23.3
	50	16	18
	25	11.3	11.3
	12.5	6.7	12.7
	6.25	8.3	8
Clorox	100	31.3	34.7
	50	24.7	27.3
	25	19.7	10.7
	12.5	13.7	5.3
	6.25	10	5.3
Hibiclens	100	42.5	35
	50	47	32.6
	25	39.3	36
	12.5	39.3	33.3
	6.25	47.3	30.6
Kaboom	100	37.3	32.7
	50	31.3	23.3
	25	20	25.3
	12.5	15.3	12.7
	6.25	6	5.3

Table 5b. *Disinfectant sensitivity tests. The resistance of MRSA was tested against disinfectants. The zones on inhibition indicate the strength of each disinfectant against MRSA.*

Disinfectant Sensitivity Tests			
		Zone of Inhibition (mm)	
Disinfectant	**Dilution (%)**	**MRSA Strain 1**	**MRSA Strain 2**
Grease Lightning	100	0	0
	50	0	0
	25	0	0
	12.5	0	0
	6.25	0	0
The Works Toilet Bowl Cleaner	100	66	68
	50	49.3	73.3
	25	35.3	40.7
	12.5	24.7	20.7
	6.25	20.7	18
Lysol 4 in 1	100	42	31.3
	50	40	32.7
	25	44	37.3
	12.5	32.7	31.3
	6.25	32.7	32.7
Rejuvnal	100	0	0
	50	0	0
	25	3.3	0
	12.5	0	0
	6.25	0	0

Table 5c. *Disinfectant sensitivity tests. The resistance of MRSA was tested against disinfectants. The zones on inhibition indicate the strength of each disinfectant against MRSA.*

Disinfectant Sensitivity Tests			
		Zone of Inhibition (mm)	
Disinfectant	**Dilution (%)**	**MRSA Strain 1**	**MRSA Strain 2**
Tilex Mold and Mildew	100	43	40.7
	50	36.7	35.3
	25	16	20.7
	12.5	18	3.3
	6.25	0	6.7
Hydrogen peroxide	100	62	47.3
	50	70	47
	25	23.3	49.3
	12.5	26.7	26
	6.25	20	13.3
70% ethanol	100	10.7	10.7
	50	3.3	9
	25	2.7	7.3
	12.5	0	3.3
	6.25	0	6

As indicated in the trials, some disinfectants and dilutions were and remained more effective than other disinfectants and their dilutions. Lysol 4 in 1 proved to be the best disinfectant by maintaining a large zone of inhibition even when diluted to 6.25%. The active ingredient in Lysol 4 in 1 was benzalkonium chloride. These results were consistent with Tilex which was very effective even when diluted to 12.5% because the active ingredient is benzalkonium chloride as well. In addition to the active ingredient

playing a role in toxicity, the pH of the disinfectant was important as well. As shown, disinfectants with either a low or high pH were the most effective. For example, The Works Toilet Bowl Cleaner had a pH of 0, and it was very effective even when diluted to 6.25%.

Although Tilex Mold and Mildew and The Works Toilet Bowl Cleaner are used in restrooms, they are still important to note because restrooms need be disinfected from MRSA as well. Lysol 4 in 1 can be used on countertops and other areas making it very useful against MRSA in the kitchen, on doorknobs, or virtually any surface as long as it is hard. Most of the disinfectants were very effective even when diluted to 12.5%, but many of the disinfectants failed to be effective against MRSA when diluted further to 6.25%.

In this study, Greased Lightning and Rejuvnal were not effective. The strains of MRSA were resistant to these disinfectants. Greased Lightning is used mainly to remove grease and stains from carpet, clothes, and countertops. Its main purpose is not to disinfect counters. Rejuvnal is a hospital-grade disinfectant. This disinfectant should have worked against both strains of MRSA. This study shows that each strain of MRSA is resistant to Rejuvnal.

Hibiclens, which is an antimicrobial hand soap used by physicians when prepping for surgery, was highly effective even when diluted to 6.25%. Povidone, which is the iodine scrub, is used to prep the surgery sites of patients. This antimicrobial scrub proved effective in the study. It is important to disinfect with antimicrobial agents that are known to be effective against MRSA considering that MRSA infections can cause a variety of illnesses based on where an infection is located in the body. Endocarditis,

urinary tract infections, and osteomyelitis are examples of infections that can be the results of contamination during a surgery procedure. If surgery sites become infected, morbidity or mortality can follow.

70% ethanol and hydrogen peroxide were effective against the bacteria as well. 70% ethanol is used in the microbiology lab at Liberty University. It was effective against MRSA, but not very effective. However, to ensure that MRSA is eliminated in the environment, 70% ethanol should not be diluted any further than the full concentration and should not be used sparingly. Hydrogen peroxide proved to be highly effective against MRSA. Hydrogen peroxide is not used to clean table tops, but has been known to clean cuts and wounds. This could be a potential chemical used to clean MRSA infections and to decontaminate surfaces.

Antibiotic Derivative Tests

The compounds exist in solid form. Because of this, they needed to be dissolved in a solvent that did not affect inhibition. DMSO and acetone were chosen to be the solvents in this experiment. The results indicated that a solvent consisting of 5% DMSO and 10% acetone should work without affecting inhibition. The results of the tests can be found in Table 6. There was no inhibition of either strain of MRSA at 10% acetone and 5% DMSO. The experiment with MRSA strain 1 will need to be completed again because some of the filter disks were contaminated with a dye called malachite green. Some dyes affect the inhibition of bacteria, which could have caused these abnormal results.

Table 6. *Solvent disinfectant tests. In preparation for testing Imidazole derivatives against MRSA, the solvents were tested first to ensure that they would not affect inhibition. The asterisks indicate that the filter disks were contaminated with malachite green.*

	Zone of Inhibition (mm)	
Solvent	**MRSA Strain 1**	**MRSA Strain 2**
H2O	12*	0
Acetone	11.3*	0
20% Acetone	6*	0
15% Acetone	2.7*	0
10% Acetone	0	0
5% Acetone	0	0
DMSO	12.7*	0
20% DMSO	3.3*	0
15% DMSO	3.3*	0
10% DMSO	3.3*	0
5% DMSO	0	0

The solid compounds were solubilized using the 10% acetone and 5% DMSO solvents. In addition, some acid or base was added to the solutions in order to fully dissolve the compounds. The molecular formulas for these compounds can be seen in Figure 5. The plates were examined after the incubation period, and it was found that all of the compounds inhibited both strains of MRSA to some degree. The results for the antibiotic derivative tests can be found in Table 7. The results indicate that the antibiotic derivatives are resistant even in smaller concentrations. The most effective antibiotic derivatives were 2-(2-chlorophenyl)-imidazole and 2-(4-chlorophenyl)-imidazole. The results coincide with the research which indicated that electron withdrawing groups and

amphipathic properties increase toxicity. This is shown primarily by 2-(4-chlorophenyl)-imidazole, which exhibits the most amphipathic characteristics out of the five compounds that were tested. According to the research studied, this compound should have been the most effective against MRSA due to its amphipathic characteristics along with the fact that it contained chlorine. Although 2-(4-chlorophenyl)-imidazole was shown to coincide with previous research, the other compounds follow the same trend with the exception of one. The chlorine groups added to the toxicity of the compound to the MRSA. All of the compounds were polar to some degree, which created an amphipathic characteristic. 2-(4-phenyl)-imidazole was the least effective in the group of antibiotic derivatives. This data is unique because the hypothesis was that 2-(4-methyletherphenyl)-imidazole would have been less effective due to less polarity.

Since each compound was effective in inhibiting MRSA, further research should be performed in order to determine if these compounds would be suitable as commercial antibiotics. The compounds could be tested with additional concentrations to determine the ideal potency as an effective antibiotic.

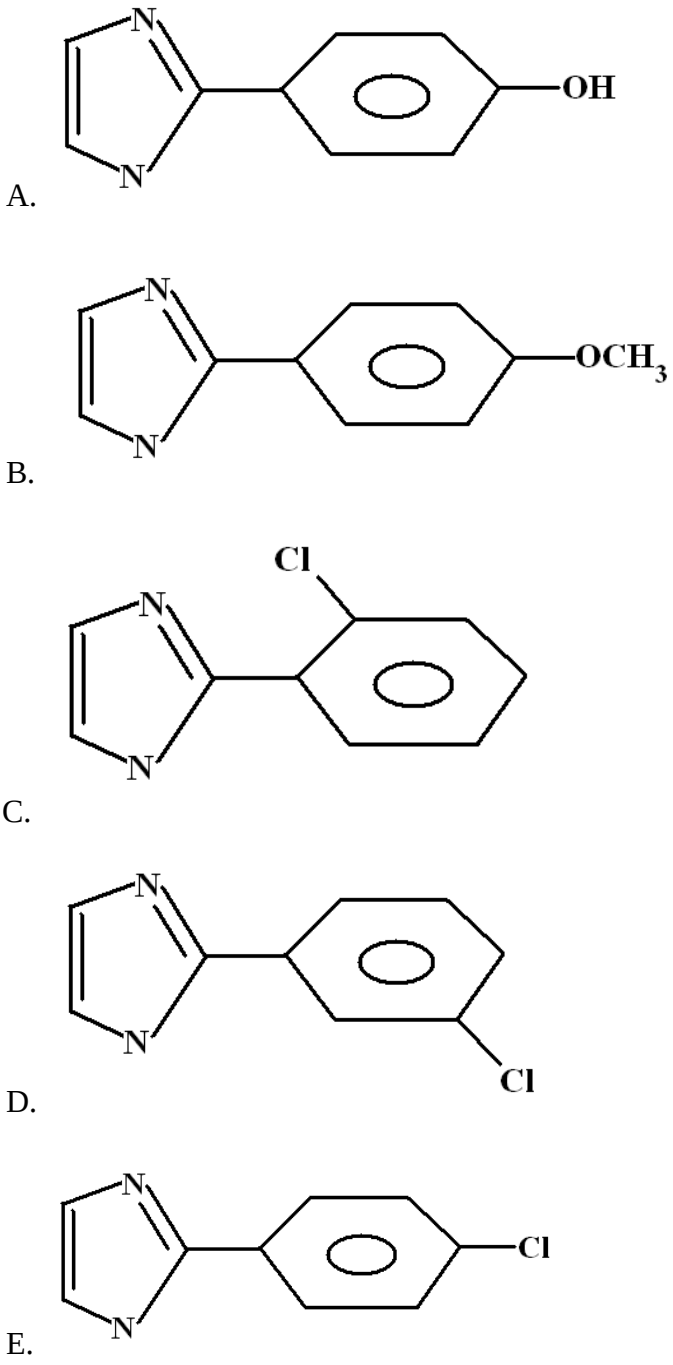

Figure 5. Molecular formulas of the antibiotic derivatives. The formulas shown are the compounds of the antibiotic derivatives tested in this study. A. is 2-(4-phenyl)-imidazole. B. is 2-(4-methyletherphenyl)-imidazole. C. is 2-(2-chlorophenyl)-imidazole. D. is 2-(3-chlorophenyl)-imidazole. E. is 2-(4-chlorophenyl)-imidazole.

Table 7. *Antibiotic derivative sensitivity test results. The compounds were tested against MRSA in four concentrations. The results indicated that each compound was effective against each MRSA strain to some degree.*

Antibiotic Derivative Sensitivity Tests			
		Zone of Inhibition (mm)	
Compound	**Concentration (mg/ml)**	**MRSA Strain 1**	**MRSA Strain 2**
2-(3-chlorophenyl)-imidazole	0.5	10	16
	0.1	8.3	10
	0.01	8	9.7
	0.001	5.3	9.3
2-(2-chlorophenyl)-imidazole	0.5	21.7	15.3
	0.1	11	9.7
	0.01	6.3	9.3
	0.001	6.3	8.7
2-(4-chlorophenyl)-imidazole	0.5	16.7	12.7
	0.1	5.3	8
	0.01	5.7	2.7
	0.001	6	6
2-(4-phenyl)-imidazole	0.5	14	9.3
	0.1	16.7	8.7
	0.01	7.3	10
	0.001	3.3	9.3
2-(4-methyletherphenyl)-imidazole	0.5	22	14.7
	0.1	11	10.7
	0.01	12.3	11
	0.001	9.7	10

Conclusion

Methicillin-resistant *Staphylococcus aureus* can be isolated from virtually any area such as in a house, daycare, or hospital. This is due to being normal flora on human

beings as well as being resistant to many antibiotics. There are two types of MRSA strains, each with distinct qualities. Hospital-acquired MRSA is found in the hospital, is known to be more resistant to antibiotic therapy, and possibly less virulent than community-acquired MRSA due to its lack of the PVL gene. Even though the chance of infection of surgery sites, wounds, or implantation of medical devices is very low, MRSA still poses a risk of prolonged morbidity and increased mortality in recovering patients. Community-acquired MRSA is found everywhere outside of the hospital, but is most often found in areas where populations interact in close quarters such as prisons, schools, and daycares. CA-MRSA is found to be more virulent since it contains the Panton-Valentine Leukocidin gene.

The antibiotic sensitivity tests revealed that Doxycycline and Streptomycin are the best antibiotics when treating these strains of MRSA. Vancomycin, Kanamycin, and Tetracycline were also highly effective against MRSA, but not as effective as Doxycycline and Streptomycin. Testing individual strains against MRSA is important when determining the best antibiotic to use, as is exhibited by the uniqueness of the two strains. MRSA strain 1 was susceptible to Ciprofloxacin but resistant to Erythromycin. MRSA strain 2 was the opposite in that it was susceptible to Erythromycin but resistant to Ciprofloxacin. A common factor in each strain of MRSA is their ability to resist β-lactams. This characteristic has been found in previous research as well as in this study. Each strain of MRSA in this study was resistant to antibiotics belonging to the β-lactam family, as is evidence by the sensitivity tests against Oxycillin, Ampicillin, and Penicillin.

It was found through the disinfectant, antimicrobial hand soap, and solution tests that many common disinfectants were effective in eliminating MRSA. Lysol 4 in 1 and

Tilex Mold and Mildew were highly effective against MRSA even when diluted to a 6.25% solution. The disinfectants each used benzalkonium chloride as their active ingredient. These two disinfectants can be used in households, prisons, schools, and daycares. The use of these products could prevent the spread of infection from MRSA as well as reducing the incidence of MRSA in the Emergency Departments of hospitals. The most commonly identified skin infection is caused by MRSA, so use of these disinfectants could reduce this statistic. The chemical structure of the active ingredient in Lysol 4 in 1 and Tilex Mold and Mildew as compared to its effectiveness correlates with other studies of antibiotics molecules in that chlorine has an active role in the toxicity against MRSA.

In the hospital setting, Hibiclens is an effective antimicrobial product for physicians prepping before surgery. This is also a helpful product to use before handling medical devices that are implanted into patients. Povidone is an effective scrub to prep patients before surgery. These two products could reduce the risk of infection from MRSA, thus decreasing morbidity and mortality in patients after surgery.

Research has shown that amphipathic molecules with electron withdrawing groups increase toxicity of antibacterial agents. Substituting with additional chlorine, carboxylic acid, or phenyl groups should increase the toxicity of these molecules against MRSA. The compounds tested in this study were shown to be effective against MRSA. Two compounds, 2-(2-chlorophenyl)-imidazole and 2-(4-chlorophenyl)-imidazole, were more polar and more effective than the other three compounds. These results coincide with previous research. Additional testing of these compounds could lead to the creation new antibiotics that will be effective against MRSA.

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