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Antibiotic sensitivity patterns of hospital-acquired and community-acquired methicillin resistant Staphylococcus aureus

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By

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ABSTRACT

Antibiotic sensitivity patterns of hospital-acquired and community acquired-methicillin-resistant *Staphylococcus aureus*

By Iyad Kaddora

Staphylococcus aureus is one of the most dangerous human pathogens. An intensive effort to control resistant staphylococci, especially methicillin resistant *Staphylococcus aureus* (MRSA), is vital as it is the most common cause of hospital-acquired infections. During the one year study period, a total of 35 MRSA isolates were collected. Fifteen isolates were identified as hospital-acquired (HA) infections, and 20 isolates were determined to be community acquired (CA). All 15 (100%) HA-MRSA strains were resistant to clindamycin and to erythromycin. Thirteen isolates (87%) were resistant to ciprofloxacin and levofloxacin, and 12 (80%) were resistant to moxifloxacin. Of the 20 CA-MRSA isolates, 15 (75%) were resistant to erythromycin, 8 (40%) were resistant to ciprofloxacin and levofloxacin, 6 (30%) were resistant to clindamycin, 5 (25%) were resistant to moxifloxacin, 2 (10%) were resistant to tetracycline, and 1 (5%) was resistant to nitrofurantion. The patterns of resistance that MRSA isolates display can play a major role in differentiating between hospital-acquired and community-acquired MRSA strains.

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Introduction

Staph infections are caused by *Staphylococcus* bacteria that are widespread in nature although they are mainly found on the skin and in the upper respiratory tracts of the host. *Staphylococcus aureus* is a Gram-positive coccus bacterium that belongs to the family Micrococcaceae. Isolated colonies of *S. aureus* are usually large (6 to 8 mm in diameter), smooth, entire, slightly raised, and translucent. The colonies of most strains are pigmented with colors ranging from cream-yellow to orange. Rare strains have relatively large capsules, which gives them a wet appearance (12). *S. aureus* is commonly found in air, dust, water, and as normal flora on skin and in the respiratory tracts of humans. The most common mode of transmission is by skin-to-skin contact from an infected host. This common bacterium is the number one cause of nosocomial infections, meaning infections that are acquired while clients are receiving care in a hospital setting (11). *S.aureus* can cause severe infections, including bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, meningitis, chorioamnionitis, and scalded skin syndrome (11). *S. aureus* can live harmlessly on the skin surface, but, when the skin is punctured or broken, it can cause serious infections that may ultimately lead to disease or even death (6, 7, 12).

Antibiotics have been successful in treating bacterial infections, but, due to overuse of antibiotics and incomplete drug courses taken by infected individuals, many clinically relevant bacteria have developed antibiotic resistance (12). In recent years, many *S. aureus* strains have acquired resistance to commonly used antibiotics. Strains

that are resistant to methicillin are common and are designated methicillin resistant *S. aureus* (MRSA). Methcillin was first introduced in 1959 and was very effective in treating patients with penicillin-resistant *Staphylococcus aureus* infections. Two years later, in 1961, the first case of MRSA was reported (6, 7). Methicillin resistance is mediated by PBP-2a, a penicillin binding protein encoded by the *mec*A gene that permits the organism to grow and divide in the presence of methicillin and other β-lactam antibiotics. The *mec*A gene is located on a mobile genetic element called a staphylococcal chromosome cassette. The relative ease of transfer of this genetic element explains the growing resistance to β-lactam antibiotics such as penicillin and its chemical derivatives as well as the cephalosporin drug (4, 6, and 7).

There are two major types of MRSA infections: health care-associated MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) (Table 1). HA-MRSA is usually associated with people who have compromised immune systems and who have had frequent or recent contact with hospitals or other long-term care facilities such as nursing homes and dialysis centers (6, 12). It is commonly transmitted *via* the hands of health care workers and is associated with severe, invasive diseases in hospitalized patients. HA-MRSA is a growing health problem in United States hospitals, increasing the total staph infections acquired in the hospitals between 1995 and 2001 from 22 to 57 percent of all nosocomial infections. Worldwide, HA-MRSA infections vary from fewer than 1 percent in Scandinavia to up to 40 percent of nosocomial infections in Japan and elsewhere in Europe (6). Many hospitalized patients are carriers of MRSA; these patients can transmit the bacterium to others without showing any disease symptoms. Patients with histories of surgical site infection, intensive care, dialysis, and those with weakened

immune systems or who are undergoing invasive medical procedures are at elevated risk of HA-MRSA infections. The ability of MRSA to form biofilms on nosocomial medical devices has increased the organism's survival and multiplication rate on these surfaces by prolonging the duration of the organism's exposure to antibiotics and promoting the potential opportunity to replicate and exchange antibiotics resistant genes (6, 12).

CA-MRSA strains are resistant to many commonly prescribed antibiotics. They are associated with skin and soft tissue infections of otherwise healthy individuals with no recent health care exposure. In early 1980s, CA-MRSA was first reported among intravenous drug users and has become the most frequent cause of skin infections in humans (6). Both HA-MRSA and CA-MRSA strains are transmitted by skin to skin contact although they have distinct clinical characteristics (6). See Table 1 for a comparison of HA-MRSA and CA-MRSA characteristics.

In clinical settings it is important to differentiate between HA-MRSA and CA-MRSA infections to help determine the most effective treatment and to reduce the rate of infections in hospitals and the community. The bacteriologic characteristics of MRSA isolates can play an important role in determining and differentiating the type of infection (1). Antibiotic susceptibility pattern analysis and the minimum inhibitory concentration (MIC) profile of each isolate represent the practical initial typing for most hospitals. Although genotyping methods are reliable and accurate, the cost prevents most hospitals from adopting this method. Genotyping methods include pulsed-field gel electrophoresis (PFGE), staphylococcal cassette chromosome (SCC*mec*) typing, and the presence or absence of cytotoxin Panton-Valentine Leukocidin gene (PVL). PVL makes MRSA more

virulent by creating pores in the membrane of infected cell host. Four major types of SCC*mec* elements have been defined based on the *mec* gene complex, which encodes methicillin resistance, and the *ccr* gene complex, which encodes the genetic recombination enzymes responsible for gene mobility (2, 3, 5). SCC*mec* carries a set of antibiotic resistance genes besides the *mec*A gene that is responsible for resistance to methicillin. Previous studies have shown that HA-MRSA strains carry SCC*mec* type II whereas CA-MRSA strains carry SCC*mec* type IV or V; however SCC*mec* type V is found in some HA-MRSA strains. Pulsed-filed gel electrophoresis (PFGE) has been used to characterize approximately 960 *S. aureus* isolates and establish a database of PFGE patterns. HA-MRSA is typified by a USA100 or USA200 PFGE pattern whereas CA-MRSA is typified by a USA300 or USA400 PFGE pattern and frequently carries the PVL gene (2, 4, 5, 10).

Historically, HA-MRSA and CA-MRSA have had significantly different clinical features, antimicrobial resistance patterns, and treatment requirements. The patterns of antimicrobial resistance can play a major role in differentiating between HA-MRSA and CA-MRSA. An early diagnosis of HA-MRSA or CA-MRSA can help the clinician prescribe the most effective antibiotic to treat each type of infection. The ability to do this can decrease the morbidity and mortality rates as well as hospital stays and treatment costs (3).

Table 1. Comparison of common characteristics of HA-MRSA and CA-MRSA isolates

Parameter	HA-MRSA	CA-MRSA
First reported	1960s	1980s
Infections	All types	Primarily skin& soft tissue
Target host	Immune Compromised Individual	Healthy Individual
Other antimicrobials	Multiply resistant	Often not multiply resistant
Predominant age group	Older age	Younger age
Resistance gene	SCC mec type II	SCC mec IV,V
PFGE types	USA 100,200	USA 300,400
Panton Valentine Leukocidin toxin	Present	Absent

Antibiotics

Antibiotics are often referred to as being either bactericidal or bacteriostatic. An antibiotic is considered to be bactericidal if it kills susceptible bacteria and bacteriostatic if inhibited bacteria can resume growth if the antibiotic is removed or degraded. The antibiotics classes and individual antibiotics described below are those that were used in this study.

The β-lactam antibiotics. All β-lactam antibiotics have the four atom β-lactam ring (Figure 1). The β-lactam group includes natural and semi-synthetic antibiotics. Members of the b-lactam group of antibiotics include the penicillins, cefoxitin, benzylpenicillin, oxacillin, ampicillin, and cefazolin.

The mechanism of bacterial cell killing is an indirect consequence of the inhibition of bacterial cell wall synthesis. Enzymes that mediate autolysis of peptidoglycan are normally present in the bacterial cell wall but are strictly regulated to allow breakdown of the peptidoglycan only at growing points. The bactericidal action of β-lactam antibiotics results from inhibition of bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins (PBPs), which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in the bacterial cell wall. The ongoing activity of cell wall autolytic enzymes results in bacterial lysis and death (15). These antibiotics are used to treat a broad spectrum of Gram-positive and Gram-negative bacteria. They are also useful to preserve the normal intestinal flora (13).

Resistance to β-lactam antibiotics occurs in bacteria that produce enzymes that degrade the β-lactam ring. The enzyme β-lactamase breaks the lactam ring and deactivates the molecule's antibacterial properties.

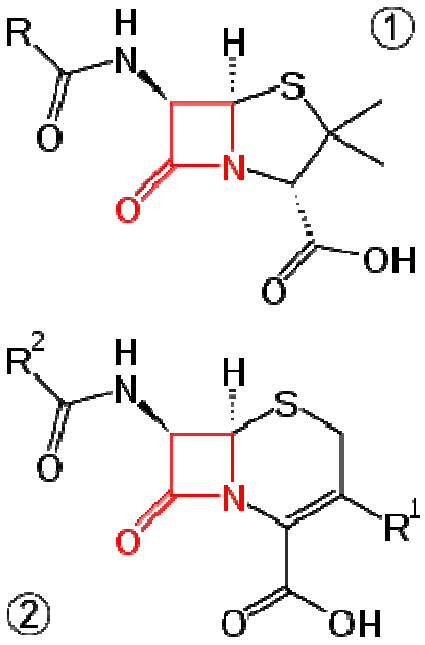

Figure 1. The molecular structure of β-lactam antibiotics; the core structure of penicillins (1) and cephalosporins (2) are shown. The β-lactam ring is shown in red.

Cefoxitin is a member of the β-lactam class of antibiotics. It is used to treat a wide range of bacterial infections that includes infections caused by Gram-positive and Gram-negative bacteria. The structure of cefoxitin is shown in Figure 2.

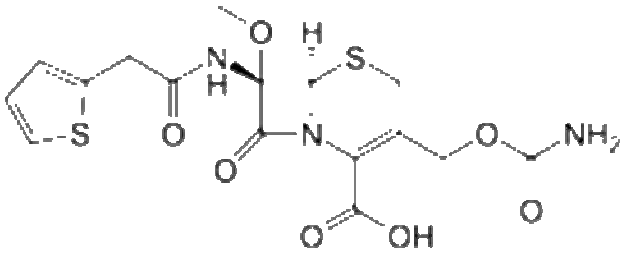

Figure 2. The molecular structure of cefoxitin.

Oxacillin belongs to the β-lactam class of antibiotics. It is widely used in treating infections caused by penicillin-resistant *S. aureus*. The molecular structure of oxacillin is shown in Figure 3.

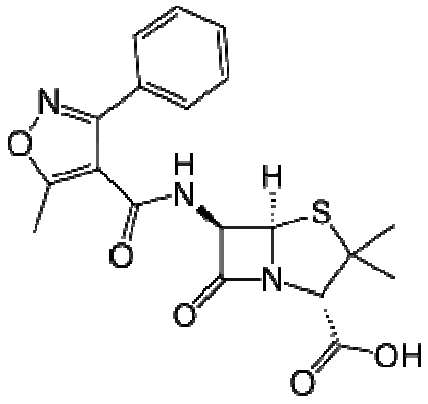

Figure 3. The molecular structure of oxacillin.

Ampicillin/sulbactam is a combination of the penicillin-derived semi-synthetic antibiotic ampicillin plus a second chemical, sulbactam, that increases the effectiveness of the antibiotic. The mixture was first used in 1987 and was commercialized in the United States under the trade name Unasyn**.** It is commonly used as an intravenous antibiotic. The second form is called sultamicillin, which is an oral form of the antibiotic marketed under the trade name Ampictam**.** Ampictam/sulbactam is used to treat infections caused by bacteria that are resistant to β-lactam antibiotics. Ampicillin/sulbactam is bactericidal. Sulbactam blocks the enzyme that breaks down ampicillin and allows the latter to kill bacteria (15).

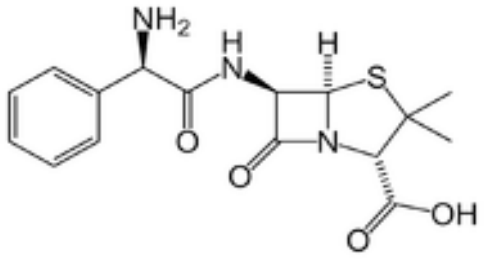

Figure 4. The molecular structure of ampicillin.

Benzylpenicillin is commonly known as penicillin G. The discovery of penicillin originated with the Scottish scientist and Nobel laureate Alexander Flemming in 1928 (15). The molecular structure of benzylpenicillin is shown in Figure 5.

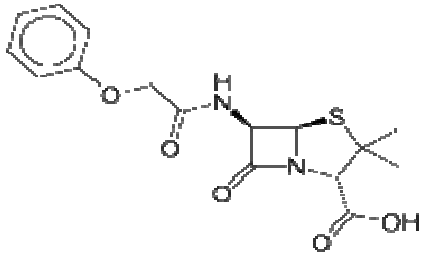

Figure 5. The molecular structure of benzylpenicillin.

Cefazolin is a β-lactam antibiotic is the first generation of a sub-class of the β-lactams called cephalosprins. It is mainly used to treat bacterial infections of the skin. It can also be used to treat moderately severe bacterial infections in the lungs, bones, joints, stomach, blood, heart valves, and urinary tract. It has proven to be effective against infections caused by staphylococci and streptococci, both of which are common on human skin. The molecular structure of cefazolin is shown in Figure 6.

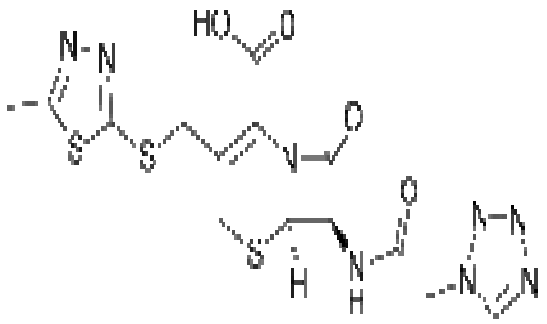

Figure 6. The molecular structure of cefazolin.

The Rifamycin antibiotics. Rifamycin antibiotics are a family of antibiotics biosynthesized by a strain of the bacterium *Streptomyces mediterranei.* The rifamycins are effective against a broad spectrum of bacteria, including Gram-positive cocci, some Gram-negative bacilli, and *Mycobacterium tuberculosis*. They are often used for the treatment of tuberculosis and for the prevention of meningococcal infections (16). Rifamycins inhibit bacterial transcription by blocking DNA-dependent RNA synthesis.

Rifampicin is a member of the rifamycin group of antibiotics. It was introduced in 1967 and is usually used to treat mycobacterial infections. Rifampicin inhibits bacterial RNA synthesis by binding to the beta subunit of DNA-dependent RNA polymerase, blocking RNA transcription (15). The molecular structure of rifampicin is shown in Figure 7.

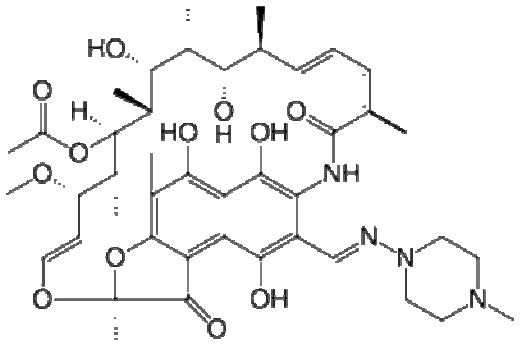

Figure 7. The molecular structure of rifampicin.

The Fluoroquinolone antibiotics. The fluoroquinolone antibiotics are characterized by the presense of a fluorine-substituted, polycyclic chemical structure. Antibiotics in this chemical class target topoisomerase enzyme that regulate supercoiling in double stranded DNA. Topoisomerases plays an important role in maintining the superhelical structure of DNA and are required for DNA replication, transcription, and transposition (15). Inhibition of topoisomerase activity results in cell death, so these antibiotics are considered bactericidal.

Ciprofloxacin is a member of the fluoroquinolone group of antibiotics. It is widely used in treating cystitis and bacterial urinary tract bacterial infections. It was approved by USA Food and Drug Adminstration and patented in 1983 by Bayer A.G. (15). The molecular structure of ciprofloxacin is shown in Figure 8.

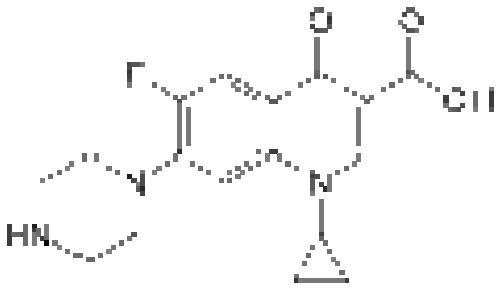

Figure 8. The molecular structure of ciprofloxacin.

Levofloxacin is a synthetic fluoroquinolone antibiotic that is used to treat life-threatening bacterial infections. It is usually sold under brand names like Levaquin and Travanic. The drug was first patented in 1987 and was approved by the United States Food and Drug Administration on December 20, 1996. The molecular structure of levofloxacin is shown in Figure 9.

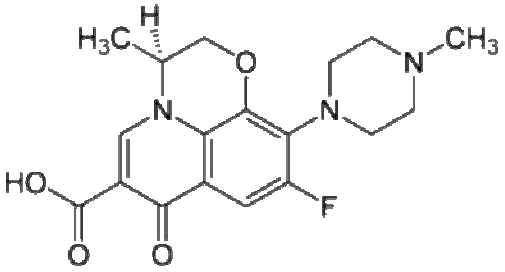

Figure 9. The molecular structure of levofloxacin.

Moxifloxacin is a third generation, synthetic fluoroquinolone chematherpautic agent developed by Bayer AG. It has been marketed under the brand names Avelox, Avalox, and Avalon for oral treatment. Avalox was submitted for approval in 1989 and

was approved in the USA for life-threatening infections in 1999. It is also marketed in 80 other countries. Moxiflocacin specifically inhibits topoisomerase II and IV, which are required for DNA replication, transcription, and recombination (15). The molecular structure of moxifloxacin is shown in Figure 10.

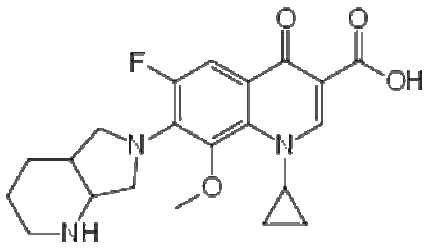

Figure 10. The molecular structure of moxifloxacin.

Gatifloxacin is sold under the brand names Gatiflo, Tequin and Zymar. It is an antibiotic of the fourth-generation fluoroquinolone family. It inhibits the bacterial enzymes DNA gyrase and topoisomeras IV. Bristol-Myers Squibb discovered Gatifloxacin in 1999 and markets the drug under the name Tequin for the treatment of respiratory tract infections (15). The sturcture of gatifloxacin is shown in Figure 11.

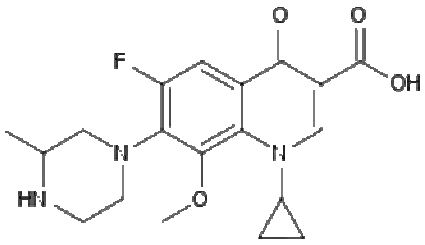

Figure 11. The molecular structure of gatifloxacin.

The Lincosaminde antibiotics. The lincosamides are antibiotics that inhibit protein synthesis by binding to the 23S ribosomal RNA molecule on the 50S subunit of bacterial ribosomes. Lincosamide binding causes premature dissociation of the peptidyl-tRNA from the ribosome leading to aborted protein translation (17). Because lincosamide binding is reversible, these antibiotics are generally considered to be bacteriostatic.

Clindamycin is a lincosamide antibiotic usually used to treat infections with anaerobic bacteria. It can also be used to treat some protozal diseases such as malaria and other bacterial infections inluding acne and MRSA. Clindamycin can be bacteriostatic or bactericidal depending on drug concentration, infection site, and the target organism (13). The molecular structure of clindamycin is shown in Figure 12.

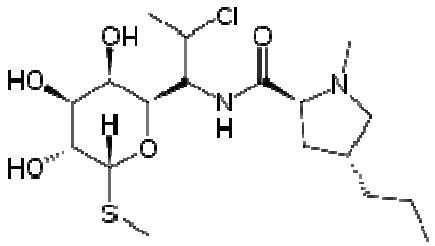

Figure 12. The molecular structure of clindamycin.

The Macrolide antibiotics. The macrolides are a group of antibiotics whose activity depends on the presence of a macrolide ring, a large macrocyclic lactone ring of 14 to 16 atoms. Macrolide antibiotics inhibit bacterial protein synthesis by preventing peptidyltransferase from adding the nascent peptidyl-tRNA to the next amino acid. They

may also inhibit ribosomal translocation and cause premature dissociation of the macrolide to the P site on the 50S subunit of the bacterial ribosome. This action is mainly bacteriostatic but can also be bactericidal if the antibiotic is present in high concentrations. Macrolides have been observed to accumulate within white blood cells, which can then transport the antibiotic to infection sites within the body (18).

Erythromycin is a macrolide antibiotic that has an antimicrobial spectrum that is wider than that of penicillin. It is also used with people who are allergic to penicllin. In addition, it is charaterized by better coverage of atypical organisms, including *Mycoplasma* and *Legionella*. Erythromycin contains a 14-membered lactone ring with ten systemic centers and two sugars, which makes it very difficult to produce by synthetic methods. The molecular structure of erythromycin is shown in Figure 13.

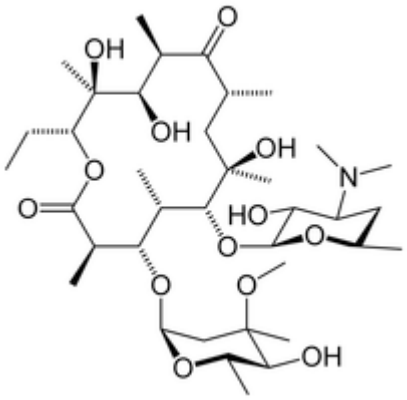

Figure 13. The molecular structure of erythromycin.

The Aminoglycoside antibiotics. An aminoglycoside is a molecule containing sugars that have been modified by the addition of one or more amino groups. Several aminoglycosides have antibiotics activity. The aminoglycoside antibiotics include gentamicin, kanamycin, neomycin, streptomycin, and several others. Aminoglycoside antibiotics interfere with bacterial protein synthesis by binding to the 30S bacterial ribosome subunit. Binding to aminoglycoside is thought to reduce the efficiency of translational proofreading, causing an increased error rate and premature termination. They may also inhibit translocation of peptidyl-tRNA between active sites on the ribosome (15).

Gentamicin is an aminoglycoside antibiotic that is used to treat many types of bacterial infections, particularly those caused by Gram-negative bacteria. However, this antibiotic is not used to treat *Neisseria gonorrhoeae*, *Neisseria meningitidis* or *Legionella pneumophila* bacterial infections. The structure of gentamicin is shown in Figure 14.

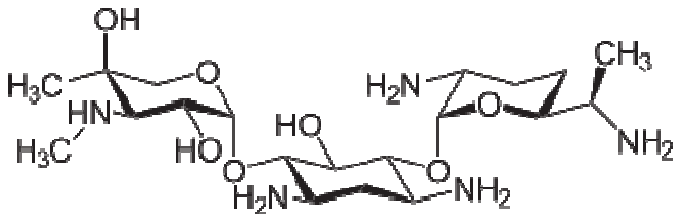

Figure 14. The molecular structure of gentamicin.

The Tetracycline antibiotics. Tetracyclines, as the name implies, are antibiotic copmpounds whose structure include four, six-member rings (see Figure 15 and 16). These compounds exert bacteriostatic activity by binding reversibly to the 30S subunit of

the bacterial ribosome and inhibiting protein synthesis. They have also been shown to cause damage to the bacterial cytoplasmic membrane (15).

Tetracycline was discovered by Dr. Benjamin Duggar in the late 1940s and is the first member of the tetracycline class of antibiotics. It is active against a wide range of Gram-positivie and Gram-negative organisms. The molecular structure of tetracycline is shown in Figure 15.

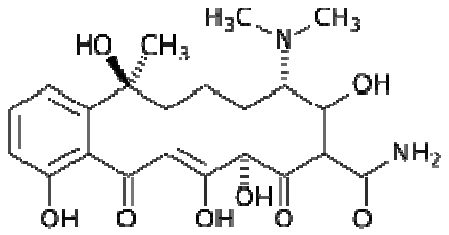

Figure 15. The molecular structure of tetracycline.

Tigecycline is another member of the tetracycline class of antibiotics. It was developed by Wheth pharmaceuticals under the brand name Tygacil. Tigecycline is a derivative of minocycline and possesses a broad spectrum activity against many clinically relevant species of bacterial pathogens including methicillin-resistant staphylococci.

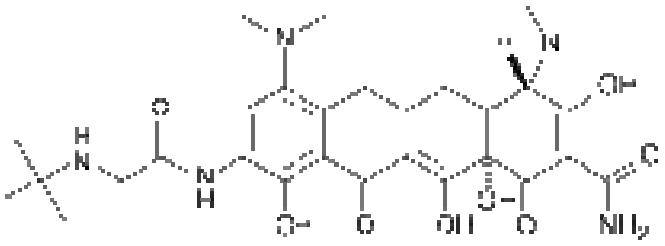

Figure 16. The molecular structure of tigecycline.

The Streptogramin antibiotics. The streptogramins are a class of antibiotics with large and complex, multi-cyclic structure. These compounds bind to the 50S subunit of the bacterial ribosome and interrupt protein synthesis by blocking the transfer of the peptidyl-tRNA and by interfering with ribosomal translocation.

Quinupristin/Dalfopristin is a mixture of two streptogramin antibiotics. The mixture has been marketed under the trade name Synercid. Each antibiotic alone is bacteriostatic, but the combination of the two shows bactericidal activity. Dalfopristin inhibits the early phase of protein synthesis by binding to the 23S ribosomal RNA molecule of the 50S ribosomal subunit. Quinupristin also binds to a site on the 50S subunit and inhibits the late phase of protein synthesis (13). Synercid is used to treat *Enterococcus faecium* and methicillin susceptible and methicillin-resistant staphylococci infections. The molecular structure of quinupristin is shown in Figure 17.

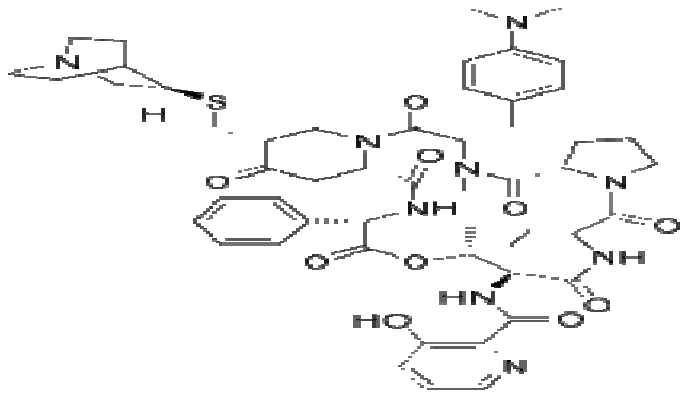

Figure 17. The Molecular structure of quinupristin.

The Glycopeptide antibiotics. The glycopeptide antibiotics are sugar-substituted cyclic or polycyclic peptides. Antibiotics of this type compromise the integrity of the cell wall in susceptible bacteria by interfereing with peptidoglycan synthesis. Glycopeptide

antibiotics bind to the amino acids in the peptidoglycan peptide cross-link and prevent the addition of new units to the peptidoglycan. Glycopeptide antibiotics are bacteriocidal. Members of this chemical class of antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin.

Vancomycin was discovered in the 1950s and was initially used to treat penicillin resistant staphylococci and other Gram-positive bacterial infections. Vancomycin binds tightly to the D-alanyl-D-alanine portion of the cell wall precursor. It also has the ability to alter the bacterial cell membrane permeability and RNA synthesis (13). The molecular structure of vancomycin is shown in Figure 18.

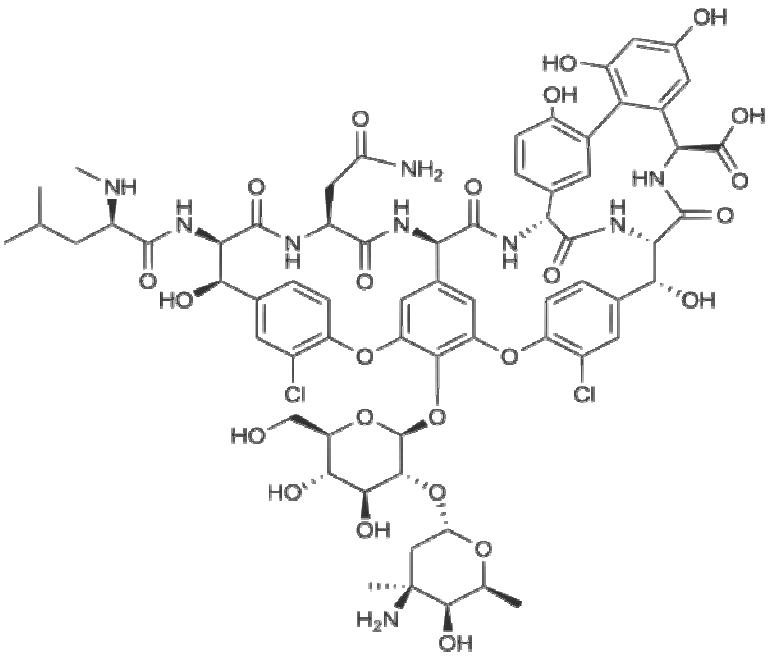

Figure 18. The molecular structure of vancomycin.

The Oxazolidinone antibiotics. Are a class of synthetic antimicrobial agents. Oxazolidinone interfer with initiation of translation. Both binding of fromlmethionine-transfer RNA to initation complexes as well as release of formylmethionine-puromycin have been reported to be targets for oxazolidinones. The binding sites of oxazolidinones are the 50S ribosomal subunits (15).

Linezolid is a synthetic antibiotic that is used to treat serious infections caused by Gram positive bacteria such as enterococci, staphylococci and streptococci. It was discovered in the 1990s, and approved for use in 2000, and was the first commercially available oxazolidinone antibiotic. Linezolid inhibits bacterial protein synthesis by binding to bacterial 23S ribosomal RNA of the 50S subunit. This prevents the formation of a functional 70S initiation complex that is essential for the bacterial protein translation process (13). The molecular structure of linezolid is shown in Figure 19.

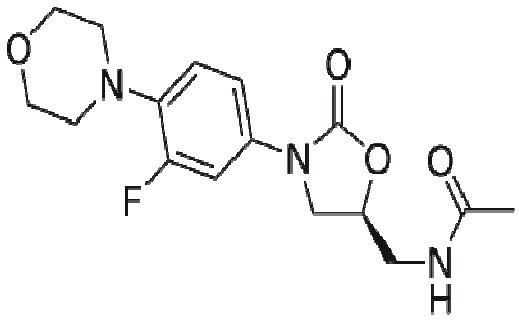

Figure 19. The molecular structure of linezolid.

Trimethoprim/Sulfamethoxazole is used to treat infections caused by Gram-negative and Gram-positive organisms. Both antibiotics are bacteriostatic agents.

Sulfamethoxazole inhibits the bacterial synthesis of dihydrofolic acid by competing with p-aminobenzoic acid (PABA). Trimethoprim inhibits dihydrofolic acid reduction to tetrahydrofolate resulting in inhibiton of dihydrofolate reductase that is required for the folic acid biosynthetic pathway that is needed for DNA synthesis and DNA repair (13, 15). The molecular structures of Trimethoprim and Sulfamethoxazole are shown in Figure 20.

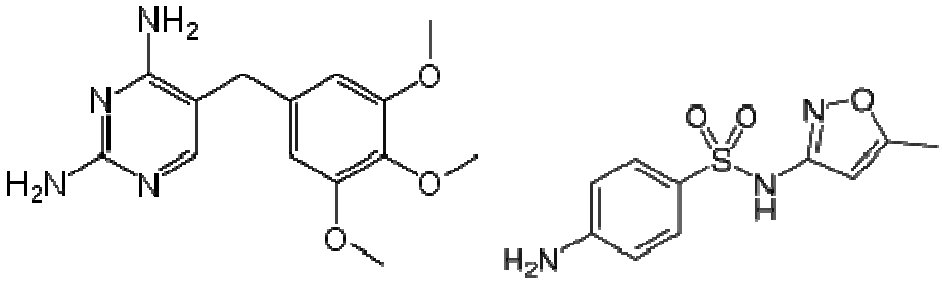

Figure 20. The molecular structures of trimethoprim and sulfamethoxazole.

The Nitrofurantoin antibiotics. Are a synthetic chemical. Nitrofurantoin is bacteriostatic in low concentration and bactericidal in higher concentration. Nitrofurantoin is reduced by bacterial flavoproteins to reactive intermediates that attack the bacterial ribosomal proteins. It also inhibits acetyl coenzyme A that is needed for metabolism and cell wall synthesis (15).

Nitrofurantoin. is used in treating urinary tract infections, specifically against *E. coli*. It is effective against *E. Coli, Enterobacter cystitis, Enterococcus, Klebsiella*, and *Staphylococcus aureus*. The molecular structure of nitrofurantoin is shown in Figure 21.

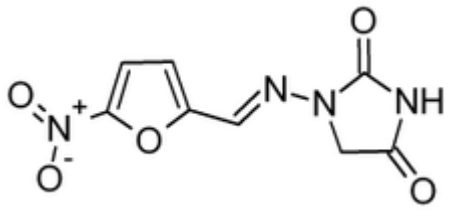

Figure 21. The molecular structure of nitrofurantoin.

Materials and Methods

Between March 2008 and March 2009, bacterial swabs were collected from patients present in clinical facilities in Huntington, WV. Some were collected *via* nares swab and the others were collected from sources such as sputum, blood culture, wound abscess, and other body fluids. Thirty-five bacterial isolates were isolated from these clinical specimens; fifteen were isolated from the nares swabs and twenty were collected from different body sites. The thirty-five isolates were presumptively identified as *Staphylococcus aureus* by performing the coagulase test, and identified to be MRSA using an automated microbiology system called the VITEK2 system.

The coagulase test is used to distinguish between different types of Staphylococcus isolates. *S. aureus* is the only species in the family that produces the coagulase enzyme, which can cause the fibrin in plasma to clot (14).

The coagulase test was done by placing one drop of sterile water on a clear slide. A sterile plastic loop was used to collect a few bacterial colonies and mix them with the

drop of water to give a heavy homogeneous cell suspension. Several loopfuls of defibrinated rabbit plasma were added to the reagent and mixed with loop. Clumping of the mixture within 10 seconds or less indicated the presence of the coagulase enzyme (14).

For each bacterial isolate, a loopful of bacteria was streaked on a new Trypticase soy agar plate with 5% sheep's blood (BAP) and incubated overnight at 37°C in a 5 % CO_2 atmosphere. Another loopful of the sample was then combined with 5 ml of Trypticase soy broth (TSB) in a test tube and incubated overnight at 37°C with 5% CO_2. Two test tubes were prepared from each sample. The test tubes were centrifuged at 1500 rpm (Beckman CS-6R centrifuge with a GH-3.7 rotor) for 5 minutes to pellet. After centrifugation, 0.5 ml of TSB/ bacterial pellet was mixed with 0.5 ml of a sterile 24% glycerol solution and placed in a sterile 4 ml glass Wheaton vial. The vial was labeled with the sample number and frozen at -70 to -80°C.

When the specimens were ready to be tested, they were allowed to sit at room temperature to thaw. The fifteen specimens that were collected *via* nares swab were streaked for isolation on MRSA chromogenic plates. CHROMagar MRSA (BD; Franklin Lakes, NJ) is a selective and differential medium, which incorporates cefoxitin for the detection of MRSA from anterior nares specimens. The rest were confirmed to be MRSA using an automated microbiology system utilizing growth-based technology called the VITEK 2 system (BIOMERIUX; Durham, NC).

Chromogenic Methicillin Resistant *Staphylococcus aureus* (MRSA) agar is designed for the qualitative, direct detection of nasal colonization by MRSA. This

selective medium was created as part of an effective program to prevent and control the spread of MRSA in healthcare settings. The test is performed on nasal swab specimens from hospital patients to screen for MRSA colonization. Colonized patients then will be targeted for isolation and appropriate treatment as early as possible.

MRSA chromogenic medium permits the direct detection and identification of MRSA through the incorporation of specific chromogenic substrates and cefoxitin. In the presence of cefoxitin, MRSA strains will grow and produce mauve colored colonies resulting from the hydrolysis of the chromogenic ingredient (Figure 22). To suppress the growth of other organism such as Gram-negative bacteria, yeast, and some Gram-positive cocci, additional selective agents were added to the medium.

Figure 22. MRSA growing on CHROMagar™

Susceptibility tests are indicated when the bacterial isolate is thought to belong to a species capable of exhibiting resistance to commonly used antimicrobial agents. Isolated colonies of an organism that may play a pathogenic role are selected from an agar plate and tested for growth in the presence of several antibiotics. Test results are then examined and the Minimum Inhibitory Concentration (MIC) for each antibiotic is

determined. The MIC helps determine the concentration of an antibiotic agent needed at the site of infection to inhibit the infecting organism.

MICs have been determined traditionally using antibiotic concentrations derived from serial twofold dilutions. The MIC is then defined as the lowest concentration of antibiotic that shows inhibition of growth. Interpretative criterion (Susceptible, Intermediate, or Resistant) can then be assigned to MIC results to aid in the direction of therapy. The standard and reference procedures are based on susceptibility tests requiring 16 to 24 hours of incubation. Various manufactures have now developed automated procedures designed to generate results more rapidly by using shortened incubation times.

The antimicrobial susceptibility testing card (AST) for the VITEK 2 system (BIOMERIUX; Durham, NC) is an automated test methodology based on the Minimum Inhibitory Concentration (MIC) technique. This card is essentially a miniaturized and abbreviated version of the two-fold dilution technique for MICs determined by the microdilution method. Each test card contains 64 microwells (Figure 23). A control well that contains only microbiological culture medium is resident on all cards with the remaining wells containing premeasured amounts of specific antimicrobials combined with culture medium.

Figure 23. Antimicrobial susceptibility testing card (AST) for the VITEK 2 system

The bacterial isolates to be tested must be diluted to a standardized concentration in saline before being used to rehydrate the card. The card is then filled, sealed, and placed into the instrument incubator/reader. The instrument will monitor the growth of each well in the card over a specified period of time, and the MIC for each antimicrobial on the card is determined at the end of this period (up to 18 hours). AST card performance may be compromised if an organism not within the appropriate range of the DENSICHEK instrument suspension is used.

The test is performed by first transferring 3.0 ml of sterile saline (0.45% to 0.5% NaCl, ph 4.5 to 7.0) into a clear, sterile plastic test tube provided by BIOMERIUX. Isolated colonies from a primary plate were selected if culture requirements were met, or the organism was sub-cultured to be tested on an appropriate agar medium and incubated accordingly. The inoculum was obtained from a pure culture; if not a re-isolation step was required before testing. Using a sterile stick, sufficient numbers of the tested organism's colonies were transferred to the saline tube. A homogenous organism suspension with a density equivalent to McFarland No. 0.50 to 0.63 was prepared using a calibrated DENSICHEK (BIOMERIUX; Durham, NC). Density was adjusted by adding

more bacteria or sterile saline as required. In a second tube containing 3.0 ml of saline, 280 µl of the adjusted cell suspension were added to the AST-GP cards. The age of suspension should not exceed 30 minutes before loading into the card. The dilution tube and AST card were placed into the instrument incubator/reader. The results were retrieved from the instrument in approximately 8 hours.

Clindamycin susceptible, erythromycin resistant *Staphylococcus aureus* may develop clindamycin resistance. Clindamycin disk inductions test (D-test) was developed to test the future resistance of *S. aureus* to clindamycin. The D-test is performed by placing clindamycin disk 15-22 mm away from erythromycin disk in blood agar plate with the tested organism. A flattening of the zone in the area between the two disks will indicate the organism's ability to induce clindamycin resistance in the future.

Results

Of the 35 isolates of methicillin resistance *Staphylococcus aureus* (MRSA), 15 strains were known to be hospital-acquired MRSA while 20 were community acquired. The first 15 were confirmed to be hospital acquired by a negative MRSA screen upon patient's admission and positive MRSA screen after the patient's discharge from the hospital. All 15 HA-MRSA strains (100%) were resistant to clindamycin, and erythromycin; 4 (27%) were D-test positive (meaning they are sensitive to clindamycin *in vitro* and resistant *in vivo*). Thirteen isolates (87%) were resistant to ciprofloxacin and levofloxacin, and 12 (80%) were resistant to moxifloxacin. Of the 20 CA-MRSA, 15

(75%) were resistant to erythromycin, 8 (40%) were resistant to ciprofloxacin and levofloxacin, 6 (30%) were resistant to clindamycin, 5 (25%) were resistant to moxifloxacin, 2 (10%) were resistant to tetracycline, and 1 (5%) was resistant to nitrofurantoin. All 35 strains (100%) were sensitive to gentamicin, quinupristin/dalfopristin, linezolid, vancomycin, rifampicin, and trimethoprim/sulfamethoxazole. All 15 HA-MRSA strains were also sensitive to tetracycline, nitrofurantion, and tigecyclin (Tables 2 and 3).

Table 2. Antibiotic sensitivity patterns of HA-MRSA

Antimicrobial	**No. of Resistant isolates (n=15)**	**% Resistance**
Benzylpenicillin	15	100%
Oxacillin	15	100%
Gentamicin	0	0%
Ciprofloxacin	13	87%
Levofloxacin	13	87%
Moxifloxacin	12	80%
Erythromycin	15	100%
Clindamycin	15	100%
Quinupristin/Dalfopristin	0	0%
Linezolid	0	0%
Vancomycin	0	0%
Tetracycline	0	0%
Tigecycline	0	0%
Nitrofurantion	0	0%
Rifampicin	0	0%
Trimethoprim/Sulfamethoxazole	0	0%

Table 3. Antibiotic sensitivity patterns of CA-MRSA

Antimicrobial	**No. of Resistant isolates (n=20)**	**% Resistance**
Benzylpenicillin	20	100%
Oxacillin	20	100%
Gentamicin	0	0%
Ciprofloxacin	8	40%
Levofloxacin	8	40%
Moxifloxacin	5	25%
Erythromycin	15	75%
Clindamycin	6	30%
Quinupristin/Dalfopristin	0	0%
Linezolid	0	0%
Vancomycin	0	0%
Tetracycline	2	10%
Tigecycline	0	0%
Nitrofurantion	1	5%
Rifampicin	0	0%
Trimethoprim/Sulfamethoxazole	0	0%

Table 4. Antibiotics resistance percentage of HA-MRSA and CA-MRSA strains.

Antimicrobial	**HA-MRSA% resistance(n=15)**	**CA-MRSA% resistance(n=20)**
Benzylpenicillin	100%	100%
Oxacillin	100%	100%
Gentamicin	0%	0%
Ciprofloxacin	87%	40%
Levofloxacin	87%	40%
Moxifloxacin	80%	25%
Erythromycin	100%	75%
Clindamycin	100%	30%
Quinupristin/Dalfopristin	0%	0%
Linezolid	0%	0%
Vancomycin	0%	0%
Tetracycline	0%	10%
Tigecycline	0%	0%
Nitrofurantio	0%	5%
Rifampicin	0%	0%

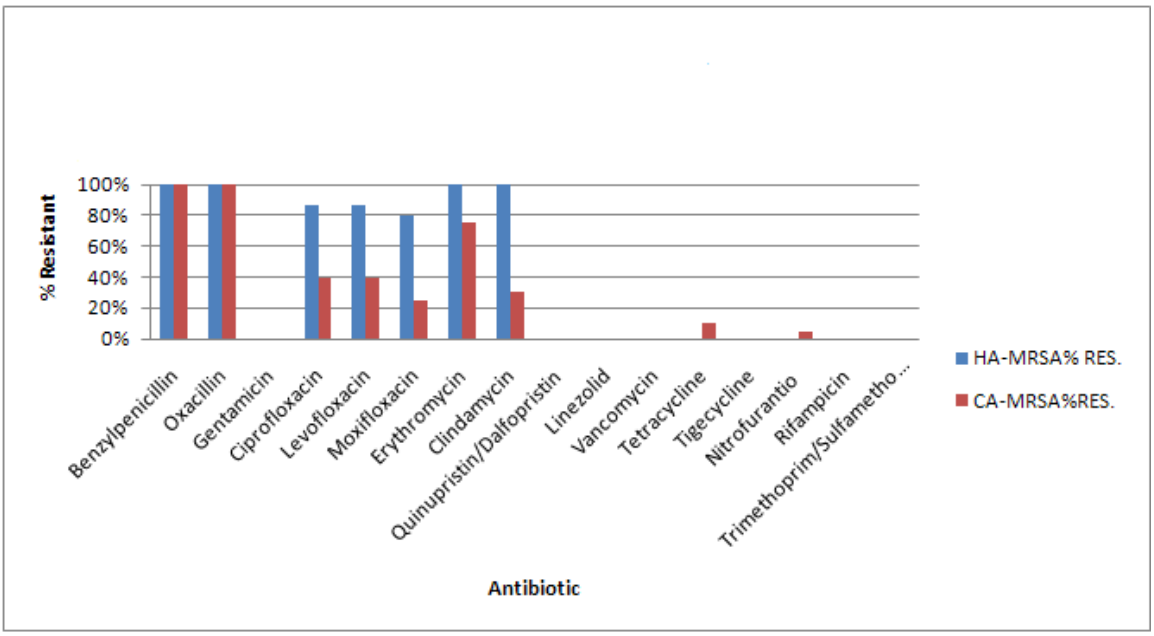

Figure 24. The percentage of HA-MRSA and CA-MRSA isolates that are resistant to each of the listed antibiotics.

Table 5. Regression models selected by chi-square score criterion.

Number of Variables	Chi-Square Score	Variables Included in Model
1	17.5000	Clinda S
2	18.6250	Moxi R Clinda S
3	19.2874	Cipro R Moxi R Clinda S
4	19.3630	Cipro R Moxi R Clinda S Nitro S
5	19.3645	Cipro R Moxi R Erythro S Clinda S Nitro S

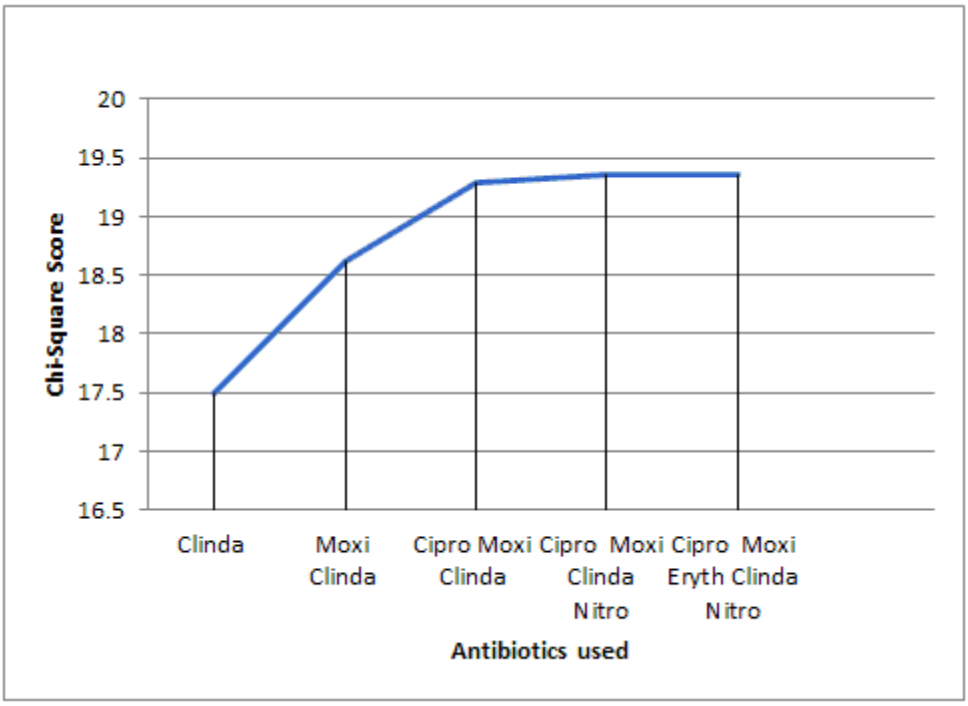

Figure 25. Chi-square score versus antibiotic resistance.

Clindamycin sensitivity was the best individual predictor for discriminating between HA-MRSA and CA-MRSA infections (Figure 25). Moxifloxacin resistance and clindamycin sensitivity together gave a better prediction, whereas the combination of ciprofloxacin resistance, moxifloxacin resistance, and clindamycin sensitivity gave the best predication in distinguishing between these two types of infection. The graph of the antibiotics used in this study versus the chi-square scores showed adding more variables to these three did not yield any significant change in HA-MRSA versus CA-MRSA discrimination for this study. The chi-square scores leveled off around 19.5 after using the three variables, indicating that additional variables did not significantly change the ability to differentiate MRSA isolates.

For the 35 isolates that were tested in this study, a hierarchical clustering analysis based on the antimicrobial sensitivity patterns was done to create a dendrogram using the average linkage between isolates (Figure 26). The key component of the analysis is repeated calculation of distance measures between isolates and between clusters once isolates begin to be grouped into clusters. The outcome is represented graphically as a dendrogram. The dendrogram produced two major clades and one outlier. From the dendrogram we can see that most of the nares isolates, which were hospital-acquired MRSA, were clustered in one major clade. Most of the community-acquired MRSA were clustered in the other major clade. Clade 1 in figure 26 includes two of 15 HA-MRSA isolates and 12 of 20 CA-MRSA isolates. Clade 2 includes 13 of 15 HA-MRSA isolates and seven of 20 CA-MRSA isolates. The data indicate that the HA-MRSA isolates form a more coherent cluster than the CA-MRSA iolates; that conclusion is also supported by the analysis shown in figure 27.

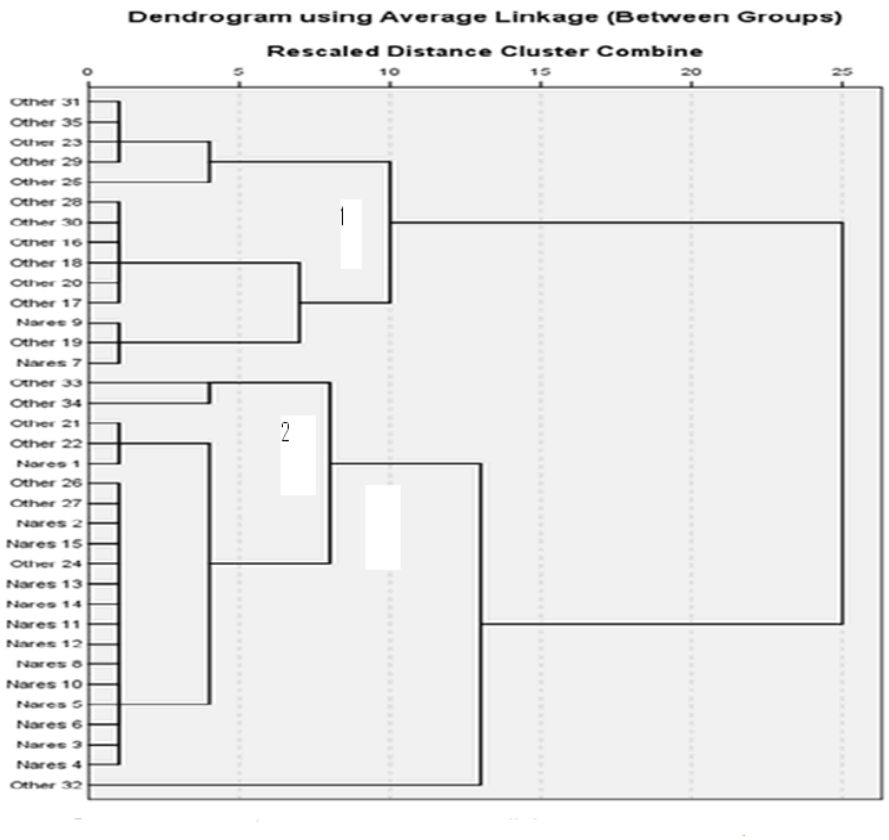

Figure 26. Dendrogram displaying cluster analysis of HA-MRSA and CA-MRSA antibiotics resistance data. Average linkage between clusters was used in producing this dendrogram. Mark clades are labeled 1 and 2.

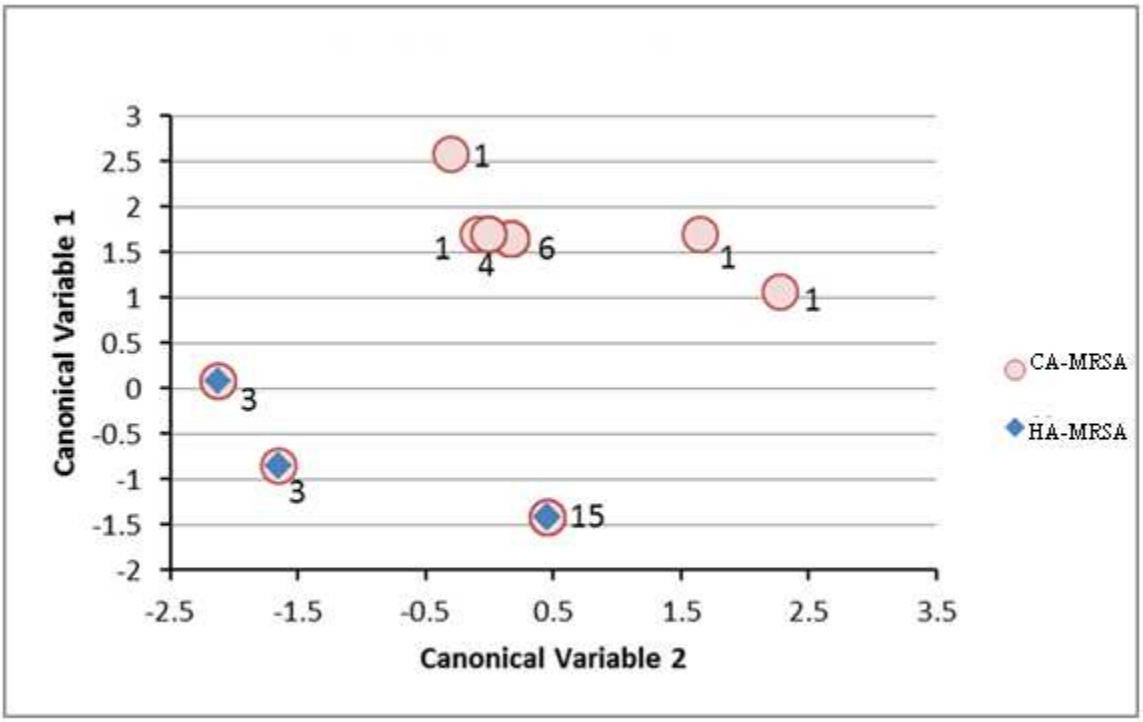

Figure 27. Graph of first two canonical variables generated by a canonical discriminate analysis of the HA-MRSA and CA-MRSA antibiotics resistance data. Numbers near the symbols indicate the number of isolates represented at each point.

Figure 27 was created by performing a canonical discriminant analysis (also called multiple discriminant analysis) on the outcome data of this study. Canonical discriminant analysis is designed to deal with variables having more than two groups. In this study having only a two-categorical variable, a simple discriminant analysis will give the same outcome. However, a canonical discriminant analysis was used for this study because it produces a two-dimensional graph. The two categories in this study are the two isolate types HA-MRSA and CA-MRSA. The graph shows that the first canonical variable alone provides as complete as possible a separation between the two categories. Drawing a horizontal line across the graph at 0.5 will show the separation between the two types of isolates, above the 0.5 line corresponds to CA-MRSA isolates, and below the 0.5 corresponds to predominantly HA-MRSA isolates. The numbers next to each

point represent the number of isolates at each point. The point at about 0 corresponds to 1 HA-MRSA isolate and 2 CA-MRSA isolates. The point at about -1 corresponds to 2 HA-MRSA isolates and 1 CA-MRSA isolate. The point at about -1.5 corresponds to 12 HA-MRSA isolates and 3 CA-MRSA isolates. As the value of the first canonical variable decreases, the percentage of HA-MRSA infections increases from 33% to 67% to 80%.

Discussion

MRSA has emerged as a serious public health problem in the United States and other regions of the world. Because of the ability of staphylococci to acquire antimicrobial resistance over time, MRSA will continue to be a problem in the future. Hospital-acquired MRSA usually causes infections in the elderly, pediatric, and immune-compromised patients, whereas community-acquired MRSA infections occur as skin and soft tissue infections in healthy individuals (6).

During the one-year study period, a total of 35 methicillin resistant *Staphylococcus aureus* (MRSA) isolates were collected. The major sources of MRSA were collected *via* the nasal cavity (15), but samples were also obtained from the abdomen (2), axilla (2), buttock (2), face (1), foot (4), inguinal (1), leg (2), sputum (3), thumb (1), and urine (2). Fifteen MRSA isolates were hospital acquired and 20 were community acquired as determined by admission and discharge screening. The antimicrobial susceptibility patterns of MRSA isolates are shown in Tables 1 and 2. Resistance to clindamycin, erythromycin, moxifloxacin, levofloxacin, and ciprofloxacin

were higher among hospital isolates when compared to community isolates. All MRSA isolates were fully sensitive to gentamicin, quinupristin, linezolid, vancomycin, tigecycline, rifampicin, trimethoprim/sulfamethoxazole, and 90-95 % sensitive to nitrofurantoin, and tetracycline.

The susceptibility of 35 MRSA strains was assessed against various antimicrobial agents using the VITEK 2 system. Clindamycin was found to be the most important antibiotic in discriminating between HA-MRSA and CA-MRSA. One hundred percent of HA-MRSA isolates were resistant to clindamycin, whereas only 30 % of CA-MRSAs were resistant to the same antibiotic. Moxifloxacin was the second most important antibiotic; 80% of HA-MRSA were resistant, and only 25 % of CA-MRSA were resistant to moxifloxacin.

Clindamycin is a unique antibiotic because isolates can be sensitive when tested *in vitro,* but some strains will become resistant when clindamycin is used in treating the infected patient. Every MRSA strain that is erythromycin resistant and clindamycin sensitive should be followed with a D test. A positive D-test indicates the ability of MRSA strains to become resistant to clindamycin during antibiotic therapy. A negative D-test indicates the effectiveness of clindamycin in treating patients with MRSA. Four of the 15 HA-MRSA strains required a D test, and all four were positive. Nine of the 20 CA-MRSA isolates were subject for a D test; however, all of them were negative. For many years clindamycin was the preferable antibiotic to be used in treating MRSA infections. This study shows that clindamycin might be effective in treating CA-MRSA but should not be used to treat MRSA that are acquired during a hospital stay.

Since the complete eradication of MRSA might not be possible, control of transmission seems to be the only hope. The first and the most effective way to control MRSA is good hand hygiene to reduce nosocomial rates of infection, along with environmental cleaning between patients. The use of broad-spectrum antibiotics for treating infections also increases the rate of MRSA and other resistant pathogens, so a more careful monitoring of antibiotics should be instituted.

Conclusion

In conclusion, this study has shown the potential for the use of antimicrobial susceptibility testing of *S. aureus* isolates, including MRSA, in distinguishing between hospital-acquired and community-acquired infections and in determining the appropriate treatment to help decrease the prevalence of MRSA and antibiotic resistance. At present, MRSA infections are treatable, but there is a need to prevent the spread of MRSA in community and hospital settings. Hand hygiene and screening health care takers and workers for the presence of these organisms will help in preventing the spread of pathogens.

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