Cantaurus, Vol. 19, 5-10, May 2011 © McPherson College Division of Science and Technology

What is the Prevalence of MRSA in a Health Care Setting Compared to a Community Setting?

Karissa Ferrell

ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a fast growing risk for many people in a health setting and in the community. Its resistance makes it hard to defeat, but with people becoming more aware of MRSA and demonstrating better hygiene, spreading it will hopefully become less in the future. I took 91 samples from McPherson College and 91 samples from a family practice clinic/hospital. I used Mannitol salt agar with oxacillin to plate my samples. The agar was a selective and differential media for the specific bacteria *Staphylococcus aureus*. The samples that grew and fermented the Mannitol salt agar and were Gram stained and isolated, then sent to Molecular Epidemiology Inc. to see if bacteria growth was positive for *Staphylococcus aureus* (MRSA). All other bacteria growth that did not ferment the agar was considered negative for MRSA. After sending in a sample to the MEI, it confirmed that 15 samples of the 91 at the family practice clinic/hospital were positive for *Staphylococcus aureus* or MRSA. McPherson College samples were compromised . Instead another student's results from an elementary school's samples were used. We found that the results were too significant to happen by chance alone. Also, surprisingly, there was a higher prevalence in the community, not the health care setting. MRSA is however still prevalent in both the health setting and in the community. Its resistance still remains strong and MRSA can be deadly if not treated. Facilities need to take cleaning seriously.

Keywords: *MRSA, Staphylococcus Aureus, mannitol salt agar, fermentation, X^2 goodness of fit test.*

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a type of bacteria that is resistant to most antibiotics, especially those in the penicillin family. These include methicillin, oxacillin, amoxicillin, and penicillin antibiotics. MRSA is a strain of *Staphylococcus aureus* that usually causes skin infections. According to the Centers for Disease Control (CDC) it appears as pustules or boils which often are red, swollen, painful, or have pus or other drainage. However MRSA can cause severe infections such as bloodstream infections, surgical site infections, or pneumonia which in some cases can be life threatening. The CDC also states that MRSA is important to study because of its pathogenicity: "MRSA have many virulence factors that enable them to cause disease in normal host." Also MRSA has limited treatment options and is transmissible. So MRSA is a dangerous bacterium that is always finding ways to evolve.

There are two types of MRSA. The first type is called health care associated MRSA or HA-MRSA. This type is acquired by being in a health care facility. Usually people who are infected are hospitalized, going in for surgery, or in a nursing home, have "an underlying chronic disease, immune suppression, or injecting narcotic use" (Del Giudice, et al, 2005). It is usually because their immune system is down or not at its best. Also many health care workers become infected by being in contact with a patient who is infected. The second type is community associated MRSA or CA-MRSA. This kind is transmitted through the community like military (bases), athletes (gym), schools, etc. Del Giudice, et al. (2005), stated that "CA-MRSA infects younger subjects and is more frequently associated with skin infections, while HA-MRSA is associated with a broader range of infections (of the urinary tract, respiratory tract, skin, etc.). CA-MRSA is more often susceptible to other antimicrobials than is HA-MRSA." So MRSA is becoming a problem because of its evolution to become resistant to many antibiotics.

Researchers in the past have tested MRSA in the health care community to determine how prevalent MRSA is. Researchers have done this in two ways. The first way is to find MRSA on patients. In the article by Mongkolrattanothai (2009) children from age's infant to 18 years old were examined. They did the "antimicrobial" therapy on those patients that tested positive for MRSA. MRSA was more prevalent in children ages four to 59 months. Their experiment also found that methicillin resistant skin and soft tissue infections have increased every year. Also in an article by Del Giudice et al, (2005) more children were infected with CA-MRSA than adults. Moran (2009) did a similar study except with adults ages 18 years and older. Again MRSA was found in patients. The majority of them had a skin infection to begin with. "About 85% of all invasive MRSA infections were associated with healthcare, and of those, about two-thirds occurred outside of the hospital, while about one third occurred during hospitalization. And about 14% of all the infections occurred in persons

without obvious exposures to healthcare" retrieved from the CDC. Unfortunately MRSA could be anywhere since in these articles people got MRSA from various places in the community or at a health care setting.

The second way to test for MRSA is testing medical equipment and/or to compare how well the cleaning methods in health care facilities do with killing bacteria. A study by Merlin, et al (2009) tested emergency personnel stethoscopes for MRSA. They found 16 of 50 stethoscopes tested had MRSA on them. Also the EMS had no idea when they were last cleaned. Another similar experiment (Chidi, 2009) tested for MRSA on medical equipment in a hospital setting. They followed the standard cleaning procedure to find if MRSA was still prevalent after being washed. Their results showed MRSA. So they put the same instruments into an automated machine and found it worked better at cleaning than manual washing. In a third study, Montgomery et al, (2010) tested for MRSA at several schools' athletic training facilities and the locker room facilities. At nine of the 10 schools MRSA was found in both facilities on at least two locations. This proves that MRSA is quite prevalent in places that may not be suspected.

These researchers have all wished they had more time to do their experiments. Testing for the prevalence of MRSA needs time especially when trying to kill the bacteria. Also many studies, like the ones done by Moran (2006) and Mongkolrattanothai (2009), wanted more range demographically to see how widespread MRSA is. So my research project would test MRSA in a family practice setting and in the community. Most experiments are done in hospitals, but a doctor at a family practice sees many patients on a regular basis. Also there aren't many studies done to see how transferrable MRSA is at a college setting among students. My research will help see the prevalence of MRSA in both types of these demographics. I will test the medical equipment that comes in contact with skin (e.g. stethoscopes, doorknobs, etc.) at the clinic and at the College I will sample comparable things like doorknobs, tables, etc. In both cases I will sample things that come in contact with human skin. In my research I will answer the question…What is the prevalence of MRSA in a health care setting compared to a community setting?

If I find MRSA at the family practice clinic and hospital I can let the doctor know so she is aware. I will also do the same for the college. So in both situations this will help them find better hygiene practices to kill the bacteria strain *Staphylococcus aureus*. Although according to Cimolai (2008), "The ability to decontaminate MRSA from environmental surfaces is an issue for debate… the efficacy of a topical agent will depend on exposure time, concentration in its solvent, humidity, temperature, and neutralization by spoilage substances, among other things. Disinfectant residue after cleaning may provide for a lingering bioactivity. Despite what may be seen as seemingly effective product and technique, the potential for prompt recontamination must be considered."

Any change will help to keep the bacteria from spreading and reaching other demographics which will limit the spread of CA-MRSA especially. My research is important to finding how prevalent MRSA is so that places such as a family practice and a college can help prevent its spread to new places and people.

MATERIALS AND METHODS

The purpose of the research is to find the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in a health care setting and compare it to how prevalent it is in the community. The research project was conducted at a family clinic and hospital in Kansas. It was chosen since it is a health setting that has many patients in and out daily. The community associated MRSA to be compared was conducted in Melhorn, the science hall at McPherson College. This building also gets many students in and out daily. There are also a few professors whose office resides in this building.

The materials that were tested included hard surfaces; at the clinic it was exam tables, at the hospital it was bed tables and in Melhorn desks were sampled. At each place the men's and women's bathrooms were tested. Each included toilet knobs, faucet knobs, soap dispensers, and door knobs. Last, at both the clinic/hospital and Melhorn two door knobs from each room were sampled. The materials needed to test, to collect, and possibly to grow MRSA were described by Ashlee Jost (personal communication, Nov. 17, 2009) included sterile Dacron swabs and sterilized Q-tips soaked in nutrient broth to collect samples, Petri dishes to hold the Mannitol salt agar with oxacillin, flasks, stir bars, and foil to make the agar, autoclave, and incubator (following making the agar), a loop, Bunsen burner, and slides to transfer bacteria, and crystal violet, iodine, decolorizing solution, safranin, and a microscope for the gram stain procedure.

The methods were also described by Ashlee Jost (personal communication, Nov. 17, 2009) including making the Mannitol salt agar, sampling, and analyzing. To begin approximately 111 grams of Mannitol salt agar was weighed and place in a flask with one liter of water followed by a stir bar. Foil was placed over the top and mixed and heated to boil for one minute. Once it was done it was placed in the autoclave for sterilization. First the autoclave needed to be filled with water to the line. Once it was the flask was put into the autoclave and shut. It was turned to steam, and then it steamed for 15 minutes at 121 degree Celsius. However because the steam

takes a bit to reach temperature, the flask stayed in for 45-60 minutes instead of the 15 minutes. It was taken out of the autoclave and put into the incubator at 50 degree Celsius for an hour to cool. Once cooled six micrograms of oxacillin was added to it, mixed slowly. Then using the laminar flow hood and turning the blower on (for sterilization purposes), the Mannitol salt agar was poured into 40 plates (25 mL each), left to cool with lid off, and then put into the refrigerator until ready for sampling.

Before samples were taken, nutrient broth was made to moisten the swabs to collect samples. Approximately 13 grams of nutrient broth was measured out and placed in one liter of water in a flask. Then a stir bar was added and it was mixed. Once mixed thoroughly the nutrient broth was put into test tubes followed by sterile swabs in each test tube. Then a cap was placed on each test tube. The test tubes in racks were placed in the autoclave for 45-60 minutes at 121 degree Celsius for sterilization. Once done the test tubes were placed in the incubator at 37 degree Celsius until ready for sampling.

The samples were taken at each location and on materials mentioned earlier. The test tubes in racks were taken to each location. The sterile technique was used to swab each sample. The swab was placed back into the test tube. When done the samples were taken to the lab. In the laminar flow hood the blower was turned on (for sterilization purposes) and the Mannitol salt agar plates were placed under the laminar flow hood with the lids off. Then the cotton swabs were taken out of each test tube and swabbed onto the Mannitol slat agar plates. Once done and everything labeled the plated samples were put into the incubator at 37*C (Ashlee Jost, personal communication, Nov. 17, 2009). The plates were watched for first sign of growth.

Next the Gram staining procedure including transferring from plate to slide was done when growth appeared on the plates. The gram stain procedure steps followed were described by Ashlee Jost (personal communication, Nov. 17, 2009). From the Mannitol salt agar the samples were transferred to a slide by using a loop and Bunsen burner for sterilization. The loop was sterilized by the fire then a swab of bacteria was taken from the plate and smeared on the slide. The loop was re-sterilized by the fire and placed under a running facet to catch a drop of water. The water was smeared on the bacteria. The loop was re-sterilized. The slide was placed over the fire to dry and kill the bacteria. Next the Gram staining procedure was done. This procedure includes using crystal violet, iodine, decolorizing solution, and safranin. Crystal violet was placed on the slide for 30 seconds then rinsed with water for five seconds. Second the iodine was placed on the slide for one minute then rinsed with water for five seconds. Third the decolorizing solution was placed on the slide for 15 seconds then rinsed with water for five seconds. And last the safranin was placed on the slide for one minute then rinsed with water for five seconds. When done the slides were patted dry and ready to be looked at under a microscope. *Staphylococcus aureus* is a Gram positive bacterium with a cocci shape. And the Gram staining will turn the gram positive bacteria purple. So all samples that were purple and cocci shape counted positive for MRSA and everything else was counted negative for MRSA. The samples that were positive for MRSA had two isolations done with a gram stain following each. This was to make sure the bacteria were gram positive and cocci shape. After the two isolations, samples were isolated a third time and sent to the Molecular Epidemiology Inc. (MEI) for genetic identification to find out if the bacteria was actually a *Staphylococcus aureus* strain.

So with all the materials and method procedures I hope that my results will answer my senior research question. If I do find MRSA in any environment setting I will bring it to the attention of the clinic/hospital and the college. Hopefully with the information they can conduct a way to have their equipment properly cleaned. This will help to decrease the spread of MRSA both in the community and health environments.

RESULTS

A total of 91 samples were collected at Melhorn and a total of 91 samples collected at the clinic/hospital. Samples were considered either positive or negative for MRSA; such as gram negative (pink) bacteria or rod shape bacteria were considered negative for MRSA and no further research was done to identify bacteria. Bacteria that fermented the Mannitol Salt agar, which started as red, turning it yellow were considered positive for MRSA. Further isolation was done to these samples. Also the Gram stain procedure was done to make sure it was gram positive (purple) and cocci shape. Other samples that had growth on Mannitol salt agar, but did not ferment the agar were considered negative for MRSA. According to the “Online Textbook of Bacteriology” website; “*Staphylococcus aureus* forms a fairly large yellow colony on rich medium.” Also from Jonathan Frye, “the *aureus* in *Staphylococcus aureus* means golden,” (Jonathan Frye, personal communication, Jan. 11, 2011). There were a total of 15 fermented (yellow) samples from the clinic/hospital. One sample from the 15 fermented group was transferred to the test tube with agar via sterile technique and sent to the MEI to make sure the bacteria is really a *Staphylococcus aureus* strain in MRSA. One sample from the negative MRSA group was also transferred to a test tube with agar and also sent in for identification. This was done on curiosity of what bacteria grew with oxacillin, but did not ferment. From

the fermented group the 15 samples were from the following places: Rm. 8 on door outside knob at Clinic (C), Women's Bathroom (WB) on Faucet Knob (FK) at C, WB on door outside knob at C, 1st Entrance door on outside knob at C, Men's Bathroom (MB) on Soap Dispenser (SD) at C, Rm. 4 on Exam Table (ET) at C, Rm. 3 door inside knob at C, Rm. 1 door inside knob at C, Rm. 106 on door outside knob at Hospital (H), MB on door inside knob at H, Rm. 118 on Toilet Knob (TK) at H, Rm. 116 on door outside knob at H, Rm. 118 on Bed Table (BT) at H, 1st rail on Right side at H, and Rm. 119 door outside knob at H.

When I received the results from the MEI it proved that the 15 bacteria samples that fermented the agar were indeed *Staphylococcus aureus*. So I can confirm that our results are positive for MRSA. No samples from the Melhorn group were sent to a geneticist since the samples were considered too old and I could not isolate live (from dead) bacteria or get a pure isolation. Also for these samples I do not know which bacteria fermented the agar. So unfortunately Melhorn samples will not be compared to. Instead I will compare my results from the family practice clinic and hospital to Kelly Green's results. Kelly Green took samples (also looking for the prevalence of MRSA) at a local elementary school. She had 43 samples out of 87 that fermented the Mannitol salt agar. She also sent a sample to the MEI. When she got the results she found that her bacteria samples were also *Staphylococcus aureus,* confirming positive for MRSA.

Comparing my results to Kelley's we used the X^2 Goodness of Fit test to see if there is a significance in finding MRSA and it was not just by random chance. In the X^2 Goodness of Fit test there are observed values and expected values. The observe values are in Table 1. Positive values are how many samples fermented the agar and have MRSA and the negative values are how many samples didn't ferment the agar at each facility. The expected values are found in Table 2. Next I plugged the observed and expected values into the X^2

Table 1. Observed Values

	(+)MRSA	(-)MRSA
Elementary School	43	44
Clinic/Hospital	15	91

Table 3. X^2 Goodness of Fit Equation (O-E)^2/O

	(+)MRSA	(-)MRSA
Elementary School	(43-28.2)^2/28.2 =7.8	(44-58.4)^2/58.4 =3.6
Clinic/ Hospital	(15-29.6)^2/29.6 =7.2	(76-61.3)^2/61.3 =3.5

Goodness of Fit equation, Sum of (O-E)^2/E (Table 3). These values added together equaled 23.03. There is 1 degree of freedom. Looking in the X^2 table with a critical value of 3.84 and a p-value of 0.05, 23.03 is close to 0.001 P-value, in the 95 percentile. This means that our results are too significant to happen by chance alone. It is significant because if MRSA was randomly distributed then the frequency of MRSA would be equal everywhere. But since it is much more frequent at the elementary school then the clinic/hospital it makes the result findings significant.

Next I found the percentages (Table 4) to compare the prevalence. The prevalence of MRSA is more prevalent in a community setting than a health care setting. However it is not known of many children from an elementary school getting a horrendous skin infection. It is because children have a stronger immune system to fight infection. It is more known of people getting it in a clinic/hospital because patients have a weak or weaker immune system. This is why hospital patients are more susceptible to getting MRSA.

DISCUSSION

MRSA is an increasing problem in the United States causing many skin infections. It is a huge crisis in the health/medical community causing "nearly 500,000 hospitalizations and 19,000 deaths a year in the United States" (Schwarz, 2011). MRSA spreads widely and fast that it is hard to treat. Many antibiotics no longer work for MRSA due to its growing resistant. But luckily now there is a study in which a vaccine is being created to prevent MRSA. The orthopaedic scientist at University of Rochester Medical center have "discovered an antibody that reaches beyond the microbe's surface and can stop the MRSA bacteria from growing, at least in mice and in cell cultures" (Schwarz, 2011). According to this article "staph-infection is the leading cause of

Table 2. Expected Values

	(+)MRSA	(-)MRSA
Elementary School	28.2	58.4
Clinic/ Hospital	29.6	61.3

Table 4. Percentages

	(+)MRSA	(-)MRSA
Elementary School	(43/87)100 =49.4%	(44/87)100 =50.6%
Clinic/Hospital	(15/91)100 =16.5%	(76/91)100 =83.5%

osteomyelitis, a serious bacterial infection of the bone" (Schwarz, 2011).In another study by the University of Florida, they tested gym equipment at

several fitness centers to see if MRSA was present. They collected 240 samples before and after cleanings, three times a day. Surprisingly, results showed no MRSA or any staph-infection causing bacteria. Kathleen Ryan believes it has to do with people being more sanitary, she states, “People right now are going around carrying their hand sanitizer in their purse and they are hand-sanitizing everything they touch. Maybe we don’t need to be quite that worried like when you go to the gym and every time you touch something it is a potential source of some horrible bug” (2011). However it is still crucial that people be cautious of what they touch.

In the Melhorn results I did not test before and after cleaning to see if the prevalence of MRSA or bacteria growth in general changed. The Melhorn building is cleaned on a daily basis. This includes all bathrooms, lecture halls, classrooms. From what I have seen rooms are vacuumed/swept and mopped, and hard surfaces are wiped down. However I do not know what kinds of products are used to sanitize surfaces. I do not know the cleaning routine(s) at the elementary.

At the family practice clinic and hospital I do not know how often the rooms are cleaned. I did observe at the family practice clinic that the nurses wiped down the exam table and pulled a new sheet over it in between patients. But I did not notice stethoscopes, hard surfaces, and door knobs being cleaned at either facility in between patients. Also I do not know how often restrooms are cleaned. I did notice the doctor at the family practice clinic did consistently wash her hands before and after examining patients.

The results from the family practice clinic and the hospital are positive for MRSA at each facility confirmed through the MEI Staphylococcus was present on the fermented plate samples. Now that I am aware of the results I will use the information to inform both health facilities that MRSA is a present issue. Hopefully receiving this information, the health facilities will take measure in fixing the problem. Though the Melhorn results are not reliable, there was still growth on several plates and some plates were fermenting the agar. The college will be informed also about taking new measures to insure the bacterial problem decreases.

Some limitations I had were not being able to sample at more locations. A broader demographic is needed to truly confirm that the community is more prevalent with MRSA. My results alone cannot say 100% that a community setting has a higher prevalence than a health care setting. Along with this more time would be needed to conduct testing at various locations. Last testing cleaning methods would have been valuable to see which work and which do not. This will help people become aware of how washing thoroughly is important to prevent spreading.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jonathan Frye for his guidance and patience throughout this whole process. I want to thank a doctor and her staffs for letting me use the family practice clinic and the hospital to collect samples. I thank McPherson College for letting me use their facilities and equipment and for funding my research. Also I thank Kelley Green for helping me and letting me use her results to complete my research. Last I want to thank Dr. Allan Ayella and the professors of the Science Department for meeting with us and pushing us to get our paper done!

LITERATURE CITED

Bacteria Absent from Gym Equip. Laboratory Equipment. Retrieved January 27, 2011. http://www.laboratoryequipment.com/News-bacteria-absent-from-gym-equip-030411.aspx

Del Giudice, P., V. Blanc, F. Durupt, M. Bes, J. Martinez, E. Counillon, G. Lina, F. Vandenesch and J. Etienne. 2006. Emergence of two populations of methicillin-resistant *Staphylococcus aureus* with distinct epidemiological, clinical and biological features, isolated from patients with community-acquired skin infections. British Journal of Dermatology 154(1): 118-124.

Cimolai, N. 2008. MRSA and the Environment: Implications for Comprehensive Control Measures. European Journal of Clinical Microbiology and Infectious Diseases 27(7): 481-493.

Chidi, O., A. Agwu, W. Akinpelu, R. Hammons, C. Clark, R. Etienne-Cummings, P. Hill, R. Rothman, S. Babalola, T. Ross, K. Carroll, and B. Asiyanola. 2009. Contamination of Equipment in Emergency Settings: An Exploratory Study with a Targeted Automated Intervention. Annals of Surgical Innovation and Research 3(8).

Merlin, M.A., M.L. Wong, P.W. Pryor, and Kevin Rynn. 2009. Prevalence of Methicillin-Resistant *Staphylococcus aureus* on the Stethoscopes of Emergency Medical Services Providers. ProQuest 13: 71-75.

Mongkolrattanothai, K., J.C Aldag, P. Mankin, and B.M. Gray. 2009. Epidemiology of Community-Onset *Staphylococcus aureus* Infections in Pediatric Patients: An Experience at a Children’s Hospital in Central Illinois. BMC Infectious Diseases 9:112.

Montgomery, K., T. Ryan, A. Krause, & C. Starkey. 2010. Assessment of Athletic Health Care Facility Surfaces for MRSA in the Secondary School Setting. Journal of Environmental Health *72*(6): 8-11.

Moran, G.J., A. Krishnadasan, R.J. Gorwitz, G. Fosheim, L. McDonald, R. Carey, and D. Talan. 2006. Methicillin-Resistant *S. aureus* Infections among Patients in the Emergency Department. The New England Journal of Medicine 355: 666-674.

MRSA Infections. 2010. Center for Disease Control. Retrieved October 31, 2010. http://www.cdc.gov/mrsa/definition/index.html.

MRSA. Google Health. Retrieved October 31, 2010. https://health.google.com/health/ref/MRSA

Schwarz, Edward, et al. 2011. Researchers Discover Vaccine Route for MRSA. University of Rochester Medical Center. http://www.laboratoryequipment.com/News-bacteria-absent-from-gym-equip-030411.aspx?xmlmenuid=51

Staphylococcus aureus, Online Textbook of Bacteriology. Retrieved January 31, 2011. http://www.textbookofbacteriology.net/staph.html