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# Airborne Staphylococcus aureus in the Biology Labs at Southern Adventist University

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Airborne *Staphylococcus aureus* in the Biology  
Labs at Southern Adventist University

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In fulfillment of Southern Scholars senior project under the supervision of Dr. Ann Foster

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**Abstract**

*Staphylococcus aureus* has been of growing interest to the scientific community in recent years. It is not only easily transferred from one person to another, but its rapidly evolving antibiotic resistance has made it increasingly difficult to treat. Once thought to be a hospital-acquired disease, *S. aureus* is now often found in the community. Studies indicate that people and clothing can act as carriers of *S. aureus* not only directly, but through airborne particles. For these reasons a research experiment was designed to determine the prevalence of *S. aureus* in the microbiology lab and anatomy and physiology lab at Southern Adventist University during the fall semester of 2014. While these labs contain similar numbers of students, many nursing students attend microbiology in their used clinical scrubs. An air sampler was used to detect the presence of *S. aureus* in the air, while sterile cloth was hung in the breathable air column to test for the airborne transfer of *S. aureus* to cloth. These samples were taken in each lab a total of six times (both in the presence and absence of students) over a period of two months. Out of a total of ten confirmed *S. aureus* bacterial colonies, six were found in the microbiology lab (all of which were in the presence of students) and four were found in the anatomy and physiology lab (two of which were in the presence of students). Additionally, two *S. aureus* colonies exhibited resistance to a variety of antibiotics and five colonies exhibited resistance to methicillin.

Based on the results of the tests performed, it can be concluded that students do spread *S. aureus* through the air at Southern Adventist University, and that the microbiology lab experiences this at a greater rate than the anatomy and physiology lab. Finally, it appears that MRSA is present in easily detectable levels in both labs.

## Introduction

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*Staphylococcus aureus* has been of interest to the scientific and medical community due to its rapid development of antibiotic resistance in the twentieth century (Enright et al., 2002). The transmission of hospital acquired, or nosocomial, methicillin-resistant *Staphylococcus aureus* is of particular concern in the in recent years (Panlilio et al., 1992). The ability of clothing and people previously exposed to *S. aureus* to generate airborne particles laden with bacteria (Boyce et al., 1993; Lidwell et al., 1975) could bring those outside of the hospital in contact with those pathogenic and antibiotic resistant strains.

*S. aureus*, a pathogenic bacterium found on human skin and in the human respiratory tract, has been studied due to its quickly adapting antibiotic resistance (Holden et al., 2004). Methicillin-resistant *S. aureus*, (MRSA), was discovered only two years after the introduction of methicillin to combat strains of *S. aureus* that had already developed a resistance to penicillin (Enright et al., 2002). Now there is concern that its adaptability and resistance transmission could quickly lead to nearly untreatable forms of *S. aureus* becoming community pathogens (Oliveira et al., 2002).

The transmission of nosocomial and resistant *S. aureus* within the hospital setting has been extensively studied (Panlilio et al., 1992). Many of these studies have focused on the spread of antibiotic resistant forms of *S. aureus* through direct patient contact (Mulligan et al., 1993). Indirect transmission is also possible, whether it be through the contact of objects containing *S. aureus* or through airborne transmission (Sautter & Wells, 1990). Research has also shown that contaminated nurses' clothing can easily redisperse strains of *S. aureus* into the air, and this may occur when the nurse has entered another room. Therefore medical and nursing staff are not only capable of transferring *S. aureus* through direct contact, but also indirectly through the

generation of airborne particles (Lidwell et al., 1975). Another common means of aerosolization of *S. aureus* is the sneeze of a carrier, who does not need to suffer from a pathogenic *S. aureus* infection themselves (Boyce et al., 1993). Nurses in particular have been found to carry so much *S. aureus* that they have been dubbed “cloud” carriers, with frequent colonizations found in their respiratory tracts, orifices, and skin (Sheretz et al., 2001)

A study of nurses found that a particular strain of MRSA sampled in the nose would commonly be found in nurses after they had treated a patient who was infected with that strain of MRSA. A transient colonization would often be present immediately after completing duties with patients known to have MRSA, but would disappear in less than twenty four hours. In some rare cases, the nurses could retain the species for longer periods. If a nurse’s nose was still colonized after twenty four hours, it was called a short-term nasal carriage, and if it persisted for another day or longer it was deemed a persistent nasal carriage (Sautter & Wells, 1990). A separate study found that an estimated 20% of people consistently harbor a certain strain of *S. aureus*, while another 60% of people periodically harbor *S. aureus* strains that change with varying frequency (Kluytmans et al., 1997). With these periods of retention and exchange of strains, it is therefore theoretically possible that a person who came in contact with a nosocomial *S. aureus* strain, such as MRSA, could create an airborne exposure of the strain far from the location of contraction. For these reasons a research experiment was set up to determine if airborne *Staphylococcus aureus* could be isolated in the microbiology and anatomy and physiology labs at Hickman Science Center at Southern Adventist University. Colonies identified as *S.aureus* were examined for antibiotic resistance. These labs were chosen because they contain similar numbers of students, but microbiology contains many nursing students while

anatomy and physiology does not. The following discussion will present data collected in 2013 and 2014.

## **Materials and Methods**

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### **Location**

The research was performed in the Hickman Science Center on the campus of Southern Adventist University, located in Collegedale, Tennessee. The microbiology lab (HSC 2305) and anatomy and physiology lab (HSC 2111) were both tested during the week while students were present in the lab to collect experimental data, and they were also tested on Saturday nights to test for the presence of *S. aureus* in the rooms after they had been empty for at least 24 hours.

### **Air Samples**

Air samples were collected with Mannitol salt agar plates placed onto a Surface Air System Super 100 air sampler. The air sampler was set for a total intake of 200 liters of air over a two-minute period and three samples were collected in each lab per test. The air sampler was placed at the front of the class and pointed out toward the center of the students. A control was also performed during which outside air was tested with the air sampler. For control tests, the air sampler was placed about fifteen feet from the building and pointed toward the parking lot.

### **Cloth Samples**

Wooden hangers were constructed and used to hold sterile cloth strips two meters from the ceiling. The wooden hangers were suspended by twine in locations over workbenches and away from the HVAC returns so that the cloth would be exposed to the student respirable air

column while remaining difficult for students to touch either intentionally or accidentally. Six hangers were placed in each lab for the duration of the experiment, and they were sanitized with alcohol shortly before each use. Fresh pairs of sterile gloves were used to pin up and take down the cloth, which was directly placed into unused, resealable plastic bags.

### **Media and Incubation**

Media used included Mannitol salts agar, Universal Growth Broth, Mueller-Hinton agar, and Mueller-Hinton broth. Aside from the temporary refrigeration of broth containing inoculated cloth strips, all inoculated broth and plates were incubated at atmospheric pressure and at 37°C.

After the cloth samples were put into labeled bags indicating room, position, and date, approximately 200 ml of sterile Universal Growth Broth was added to each bag and they were placed in the refrigerator overnight. The next day, the bags were incubated on a shake table at 37°C for three hours. The bags were agitated a final time by hand before three 1 ml samples were plated onto three separate labeled Petri dishes containing Mannitol salt agar. The Mannitol salt agar plates, whether from the air sampler or the inoculated broth, were incubated at 37°C for 36 hours.

### **Gram Stain and Slant**

After the 36-hour incubation period, the Petri dishes were examined. The colonies that fermented mannitol were counted. Each plate that contained a colony positive for mannitol fermentation was set aside, and an isolated colony was randomly selected for Gram staining. The Gram staining procedure can be found in Appendix B. The colonies that appeared to be Gram-positive cocci were transferred onto slants and stored in a refrigerator.

### **Staphylococcus aureus ID Test**

After all samples were collected and slanted, they were tested using Fisher HealthCare's Sure-View® Color Staph ID kit. When exposed to *S.aureus*, the reagents would turn blue with red aggregates (as seen in Appendix D) to indicate a positive result.

### **Determining Antibiotic Resistance**

A loop full of each colony that was confirmed to be coagulase positive *S. aureus* was then placed into a 250 ml Erlenmeyer flask containing 50 ml of Mueller-Hinton broth. After incubating on a shake-table for three hours, samples from each beaker were spread onto three Mueller-Hinton agar plates using a sterile cotton swab. Next, a Becton Dickinson Sensi-Disc™ dispenser was used to evenly place the following small antibiotic discs on the agar: vancomycin, trimethoprim, ciprofloxacin, oxacillin, amoxicillin/clavulanic acid, clindamycin, doxycycline, and ceftriaxone. The plates were incubated for 36 hours, after which clear zones of inhibition were seen (See Appendix D). Antibiotic resistance was then determined by comparing the diameter (or twice the radius) of the zone of inhibition with previously established data. The *S. aureus* colonies were also plated onto BBL™ CHROMagar® MRSA II plates to test the colonies for methicillin resistance. The plates were divided into quadrants, and an individual colony was smeared into each quadrant. After incubation, a mauve color indicated the colony was MRSA.



## Results

The research was successful at providing a comparison of the prevalence of *Staphylococcus aureus* in the microbiology lab and anatomy and physiology lab at Southern Adventist University. All of the methods described above were used to select *S. aureus* and determine antibiotic resistance. Based on the results of the experiment, it can be concluded that there are more airborne *S. aureus* bacteria when students are in the building than when they are not (See Figure 1), and also that on those days there is a higher concentration of airborne of *S. aureus* in the microbiology lab (Figure 2). One air sample from the microbiology lab and one air sample from the anatomy and physiology lab exhibited multiple antibiotic resistances. Also, three *S. aureus* colonies from the microbiology lab and two from the anatomy and physiology lab were confirmed to be MRSA.

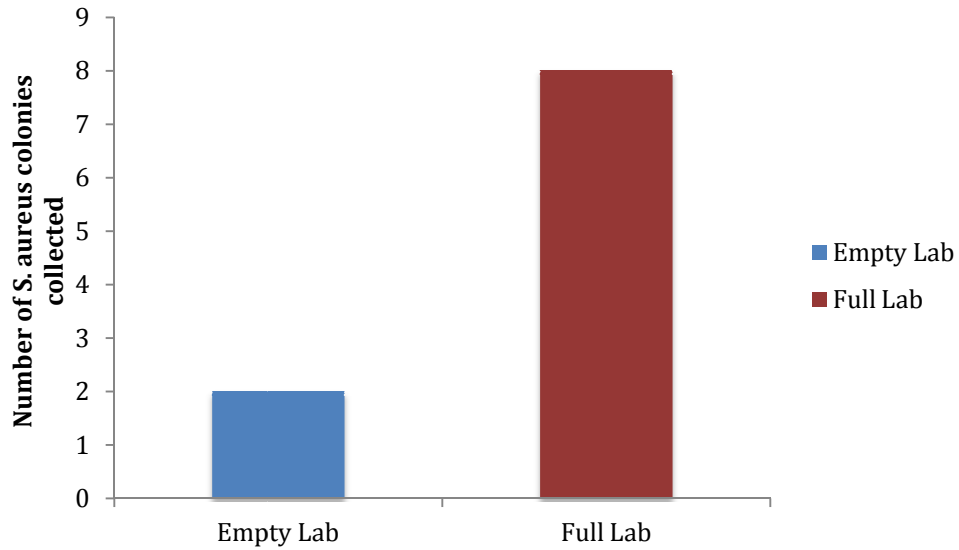


Figure 1: Ratio of *S. aureus* found in the empty labs on Saturday night to the full labs during the weekday. The numbers represent the total collected from both labs from all collection dates.

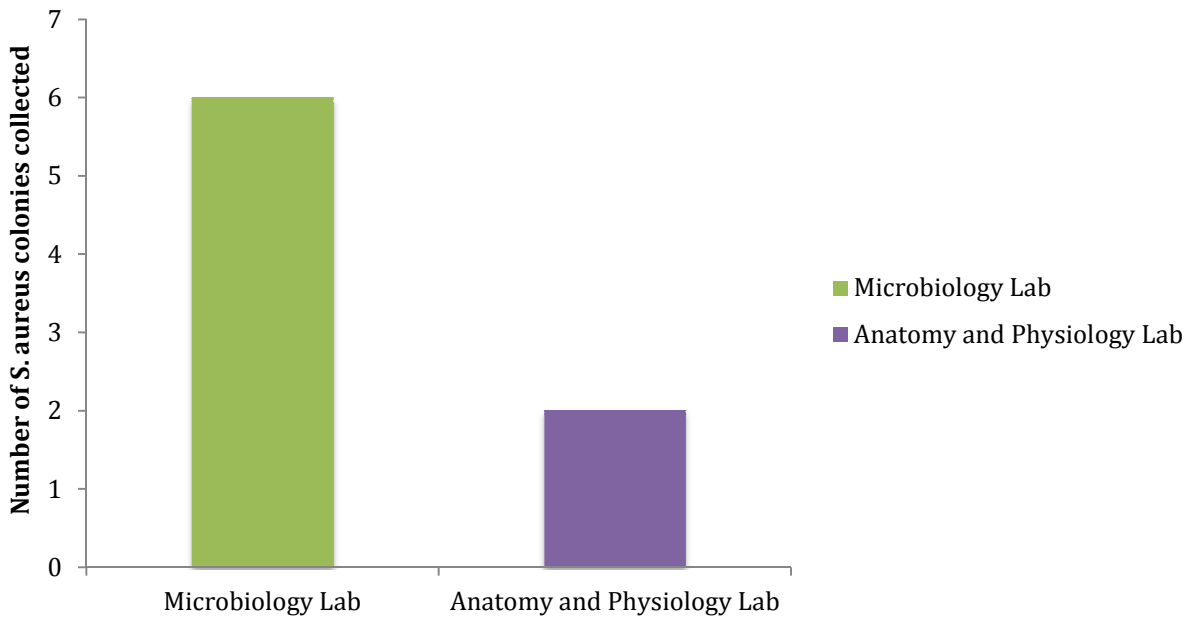


Figure 2: Ratio of *S. aureus* found in the microbiology lab to those found in the anatomy and physiology lab when only accounting for the days with students in the labs. Numbers represent a total from all collection dates during which students were present.

### Antibiotic and Methicillin Resistance

Sample	Resistant to	Intermediate to
Air Sample from Empty Anatomy and Physiology Lab on 11/02/2013	Oxacillin Clindamycin	Ceftriaxone
Air Sample from Full Microbiology Lab on 11/05/13	Oxacillin Clindamycin Ceftriaxone Methicillin	Amoxicillin/ Clavulanic acid
Cloth Sample from hanger #3 Full Anatomy and Physiology Lab on 11/06/13	Methicillin	
Cloth Sample from hanger #3 Full Anatomy and Physiology Lab on 11/06/13	Methicillin	
Air Sample from Full Microbiology Lab on 11/12/13	Methicillin	
Cloth Sample from hanger #5 Full Microbiology Lab on 11/12/13	Methicillin	

Table 1: This table contains the two *S. aureus* colonies that demonstrated antibiotic resistance and lists each antibiotic that they were able to resist including methicillin. The samples not listed showed no antibiotic resistance.

## Discussion

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In this experiment, each process was carefully planned to eventually isolate airborne *S. aureus* and determine its antibiotic resistance. First, the sterile cloth and air samplers served to collect large amounts of airborne bacteria nonspecifically. Next, the high salt concentration of the Mannitol salt agar helped to select only bacteria of a few species, one of which is *S. aureus*. Through Gram staining and microscopic examination, the field was further narrowed by selecting colonies that exhibited a deep purple color associated with Gram-positive coccus. Finally, the Sure-View® Color Staph ID kit was the final confirmation in *S. aureus*'s identity. Once a colony's identity had been confirmed, it was placed on Mueller-Hinton agar because it is a non-selective agar with good diffusion, which makes it excellent for antibiotic testing. Finally, plates of CHROMagar were used to determine methicillin resistance. A flow chart depicting the process of isolating *S. aureus* and testing for antibacterial resistance can be found in Appendix E.

One key question this research hoped to solve was if the students would significantly increase the number of airborne *S. aureus* colonies. This was confirmed, as only two colonies of *S. aureus* were collected from the combined three Saturday nights of testing while eight colonies were collected during the three weekdays of testing.

A second question that was resolved was whether there is a difference between the levels of airborne *S. aureus* in the microbiology lab and the anatomy and physiology lab. Although both contained approximately 30 students, met in the mid-afternoon, and lasted approximately the same amount of time, there were three times as many colonies of *S. aureus* collected from the microbiology lab on days when the students were present. Further research should be conducted into why this is and whether it relates to the uniforms that some nursing students wear into the

microbiology lab. Additionally, the *S. aureus* could be carried in the nasal passages of these nursing students.

The final goal of the experiment was to determine antibiotic resistance in any *S. aureus* colonies collected. Although not enough colonies exhibited antibiotic resistance to make a conclusive statement comparing the microbiology lab with the anatomy and physiology lab, their presence in Hickman Science Center has been confirmed.

This research is important because it not only indicates that students spread *S. aureus* in the air at Hickman Science Center, but it is also evidence for an unequal distribution of *S. aureus* between the labs. Furthermore, the existence of antibiotic resistance *S. aureus* especially MRSA in Hickman Science Center warrants further investigation to determine what action, if any, should be taken.

It is imperative to continue this research in order to further understand the sources and concentrations of *S. aureus* that is found at Southern Adventist University along with the risks that they could pose. Additional research could include a more thorough examination of nursing students in an attempt to connect the *S. aureus* strains on campus with an area hospital. Alternatively, other areas of campus outside of those visited by nursing students could be tested for the presence of airborne *S. aureus*.

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## Appendix A

### Wooden Hangers Used to Suspend Sterile Cloth

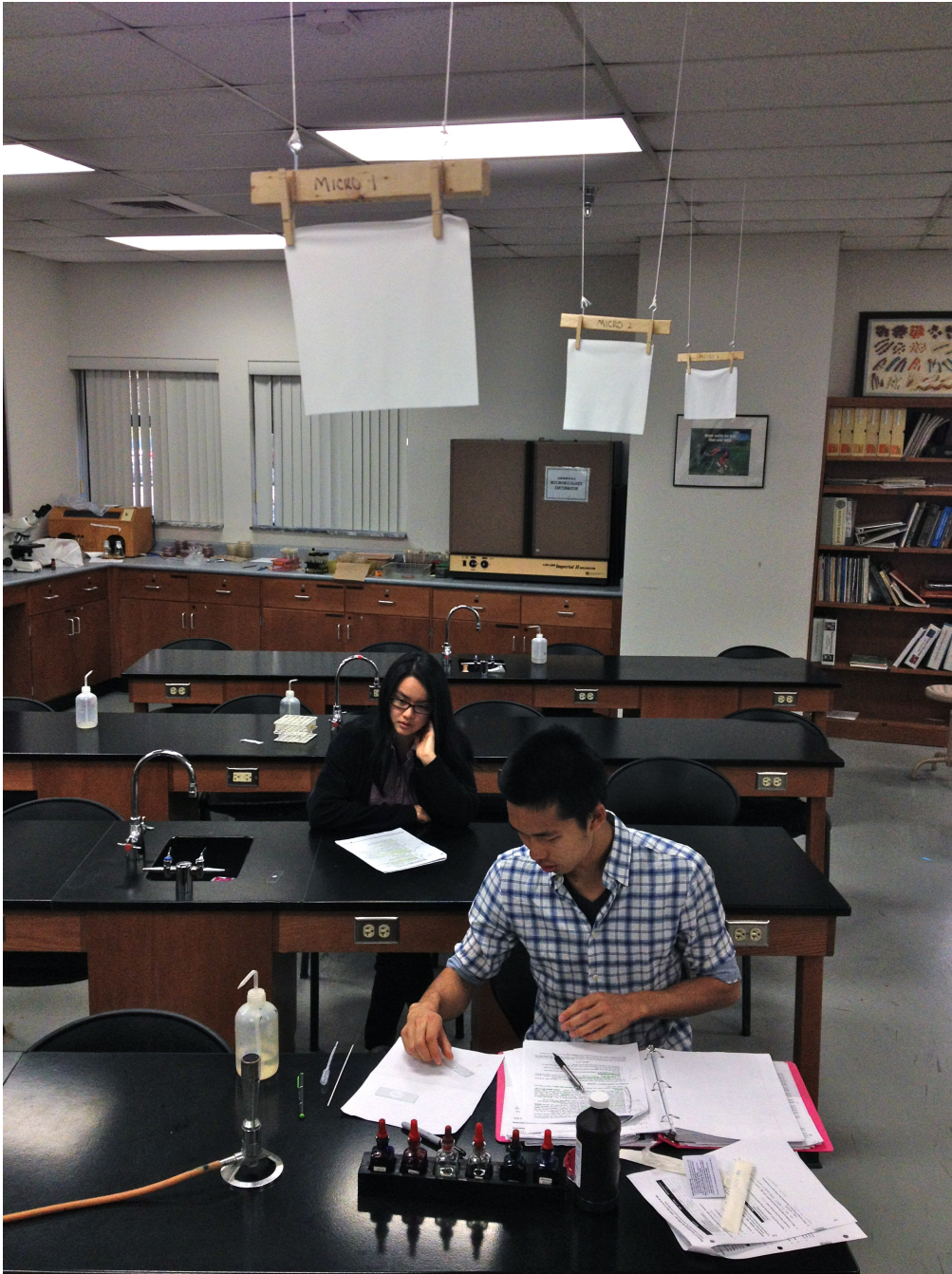


Fig 3: The wooden hangers were suspended over the lab benches in such a way as to be exposed to student breath while avoiding the HVAC returns.



## **Appendix B**

### Gram Staining Procedure

1. Place a small droplet of water on a labeled slide.
2. Gently mix desired bacterial colony into the water droplet.
3. Use a Bunsen burner to fix the smear on the slide.
4. Place the slide onto a staining tray.
5. Flood the smear with Crystal Violet solution and wait 60 seconds.
6. Gently rinse the slide with water.
7. Flood the smear with Iodine and wait 60 seconds.
8. Gently rinse the slide with water.
9. Decolorize the smear with alcohol for 15 seconds.
10. Gently rinse the slide with water.
11. Stain with Safranin for 30 seconds.
12. Gently rinse the slide with water and gently blot dry.
13. Observe under oil immersion.

**Appendix C**

Color Staph ID test

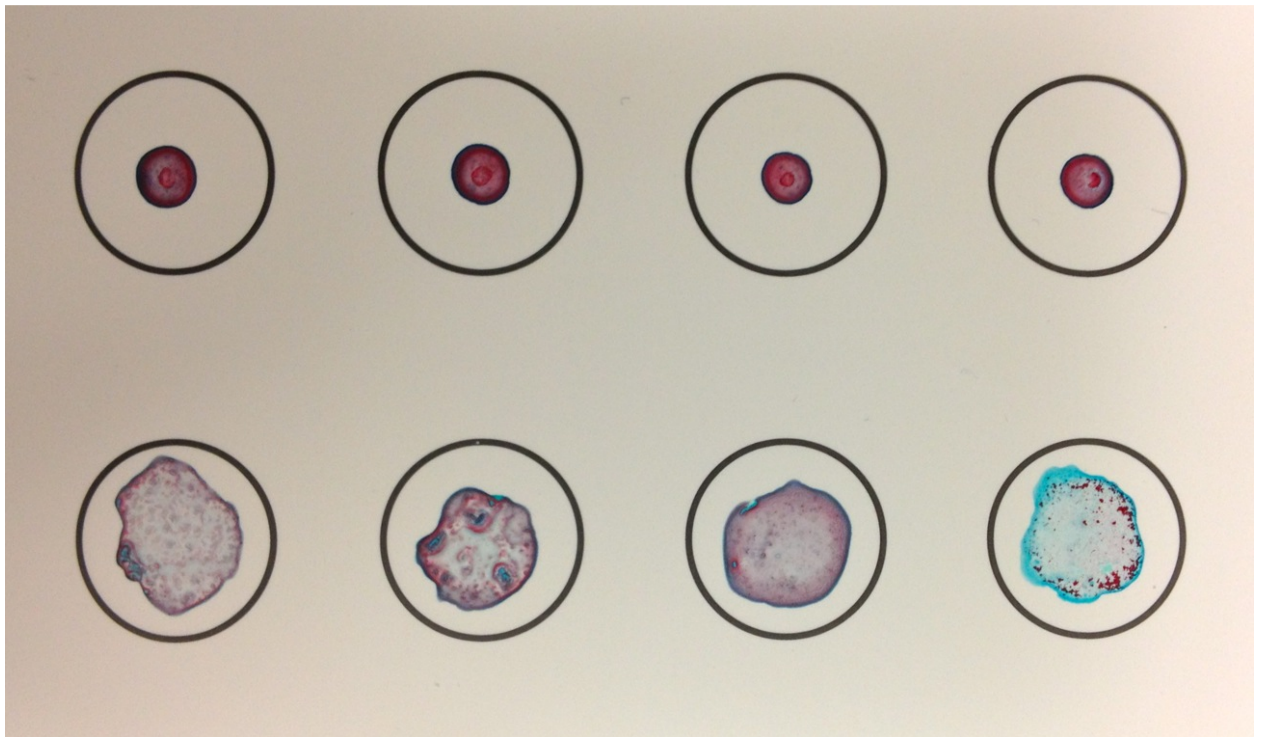


Figure 4: This picture shows a positive result for the Color Staph ID test in the farthest right sample on the bottom row. The other three on the bottom row indicate a negative result.

The top row contains controls to ensure that a false positive is not recorded.

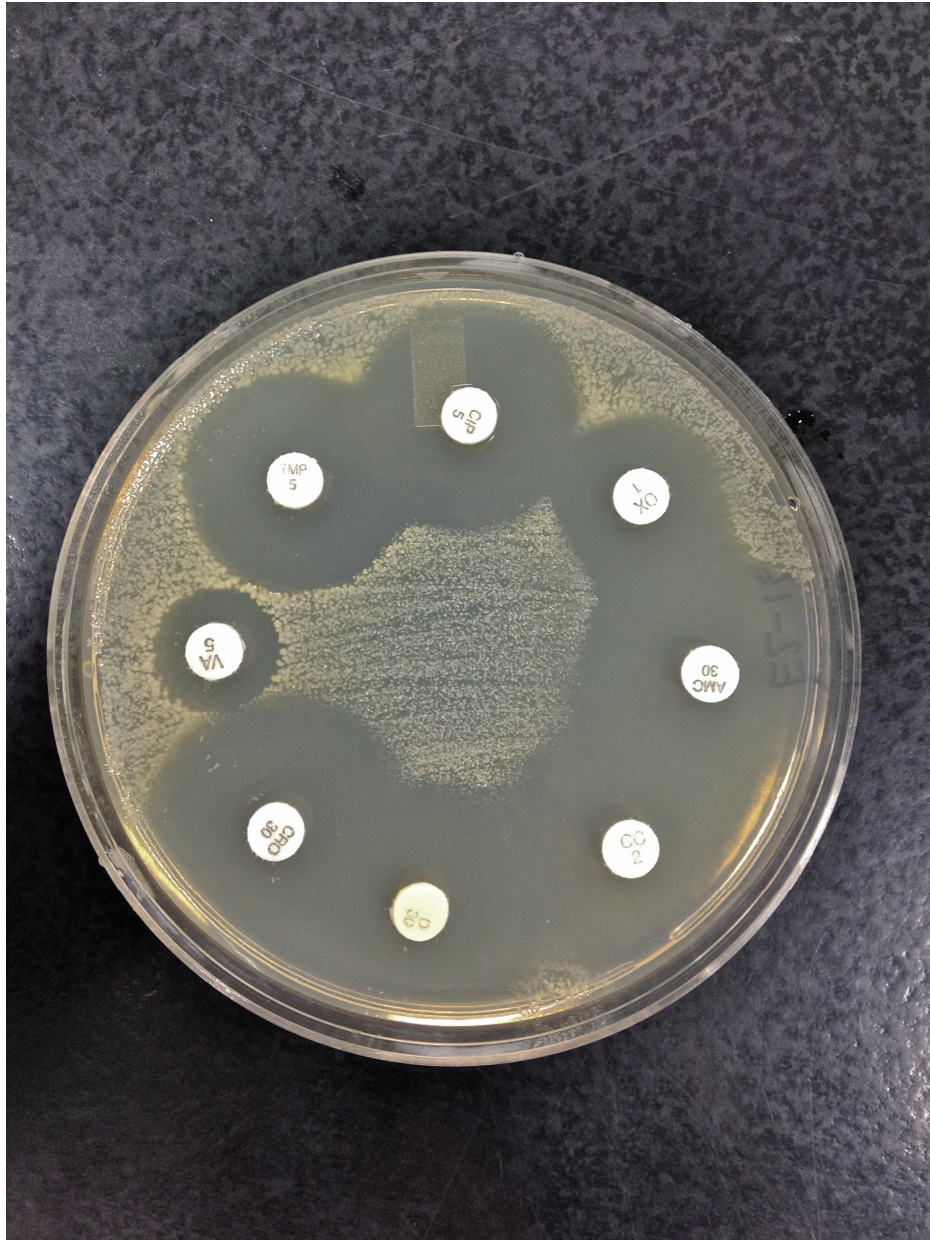
**Appendix D****Antibiotic Resistance Test**

Figure 5: This picture of eight small antibiotic chips lay atop Mueller-Hinton agar was taken at the end of its 36-hour incubation. Varying sizes of zones of inhibition surround each chip demonstrating its effect on the *S. aureus*.

Appendix E

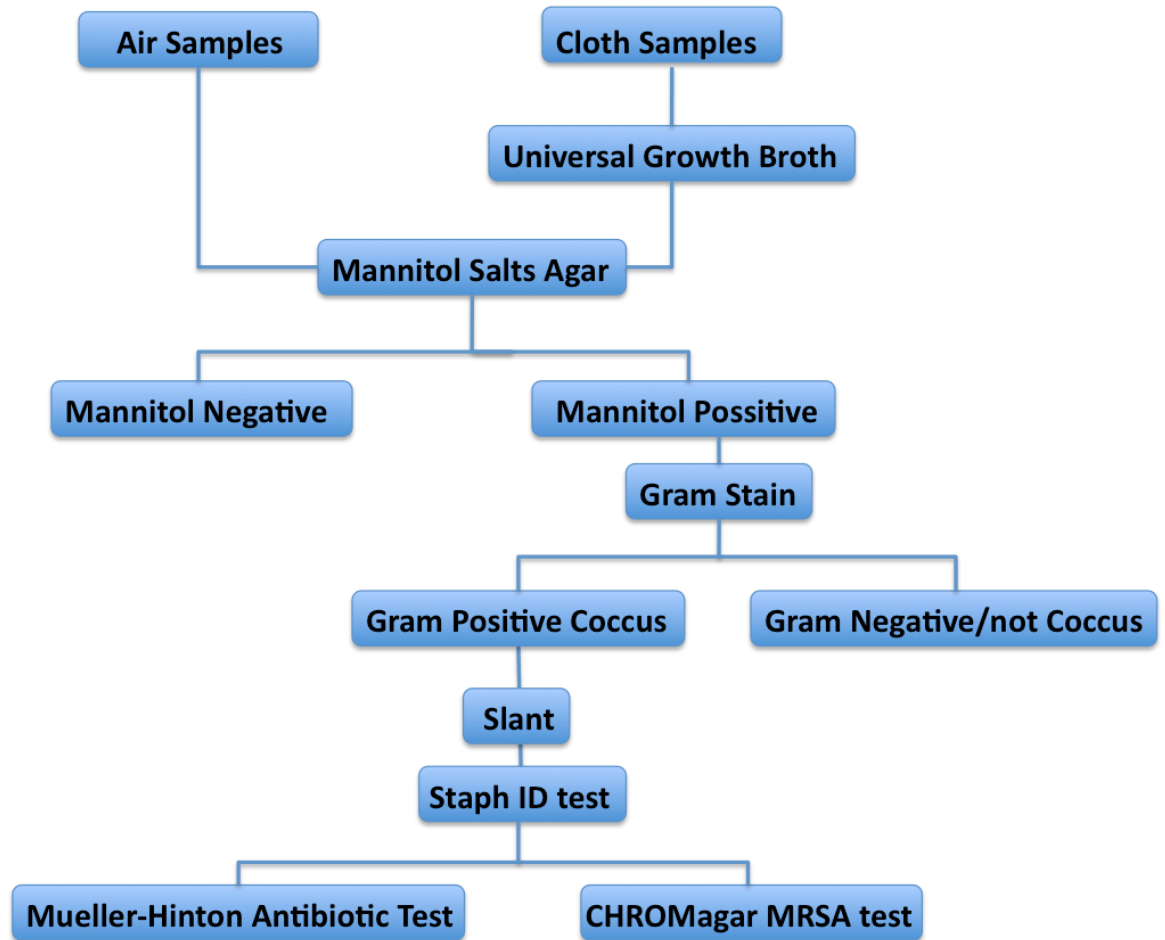


Figure 6: A flow chart depicting the overlying experimental direction including the eventual isolation of confirmed *S. aureus* and testing for its antibiotic resistance.