Characterization of Staphylococcus Aureus Isolated from the Nasal Cavity Flora of Nursing Majors

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Characterization of Staphylococcus Aureus Isolated from the Nasal Cavity Flora of Nursing Majors

Abstract
Approximately 30% of people have the bacterium Staphylococcus aureus (S. aureus) in their nasal passages. Within this group, approximately 1-2% are colonized with methicillin-resistant S. aureus (MRSA), although many do not display any symptoms.1 MRSA is an opportunistic pathogen that can potentially cause diseases such as pneumonia, skin infections and sepsis. MRSA infections are commonly grouped into two categories, hospital associated (HA) or community associated (CA) based on where the infection was acquired and the profile of antibiotic resistance. S. aureus and MRSA can spread between individuals through physical contact and presents a serious health hazard to patients if healthcare professionals are carriers. This research focuses on the detection and characterization of S. aureus strains found among a population of healthy nursing majors in multiple sections of a laboratory course at St. John Fisher College. Samples collected from the nasal passages of students were characterized using mannitol salt agar, Gram-staining procedures, blood agar, CHROMagar MRSA II and were subjected to antibiotic resistance testing. Based on the findings of this experiment, it is clear that S. aureus is present in healthy individuals. Results from the spring and fall trials demonstrated that S. aureus was present in 31% and 50% of the sample population respectively. Despite only one strain testing positive for MRSA, many other strains did exhibit antibiotic resistance similar to that of HA-MRSA. Our results reveal a vast array of S. aureus strains present in healthcare workers and support the argument that there needs to be increased awareness and policies to help prevent the transmission of infection to patients.

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Characterization of *Staphylococcus aureus* isolated from the nasal cavity flora of nursing majors

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### Abstract

Approximately 30% of people have the bacterium *Staphylococcus aureus* (S. aureus) in their nasal passages. Within this group, approximately 1-2% are colonized with methicillin-resistant *S. aureus* (MRSA), although many do not display any symptoms. MRSA is an opportunistic pathogen that can potentially cause diseases such as pneumonia, skin infections and sepsis. MRSA infections are commonly grouped into two categories, hospital associated (HA) or community associated (CA) based on where the infection was acquired and antibiotic resistance. S. aureus and MRSA can spread between individuals through physical contact and presents a serious health hazard to patients if healthcare professionals are carriers. This research focuses on the detection and characterization of *S. aureus* strains found among a population of healthy nursing majors in multiple sections of a laboratory course at St. John Fisher College. Samples collected from the nasal passages of students were characterized using mannitol salt agar, Gram-staining procedures, blood agar, CHROMagar MRSA II and were subjected to antibiotic resistance testing. Based on the findings of this experiment, it is clear that *S. aureus* is present in healthy individuals. Results from the spring and fall trials demonstrated that *S. aureus* was present in 31% and 50% of the sample population respectively. Despite only one strain testing positive for MRSA, many other strains did exhibit antibiotic resistance similar to that of HA-MRSA. Our results reveal a vast array of *S. aureus* strains present in healthcare workers and support the argument that there needs to be increased awareness and policies to help prevent the transmission of infection to patients.

### Introduction

Microorganisms known as normal microbiota, or flora, colonize the human body and are essential to human health. *Staphylococcus aureus*, abbreviated as *S. aureus*, is commonly found as part of the normal flora on the skin and within the respiratory tract. As of 2011, approximately 30% of people in the United States were colonized with *S. aureus*. Although *S. aureus* is not always pathogenic, it can cause many different types of infections and is a significant cause of infections contracted while within a healthcare facility. From 1997 to 1999, *S. aureus* was reported as the leading cause of infections in the blood, skin, and respiratory tract, requiring over $14.3 billion for treatment.

This bacterium employs a variety of virulence factors in addition to drug-resistance causing a high mortality rate of about 20%. *S. aureus* is able to avoid phagocytosis in the body by camouflage themselves with specialized protein coats or by forming a biofilm. It also has the capability of secreting chemicals that interfere with receptors of phagocytes and can lyse them leading to a higher survival rate. These characteristics make *S. aureus* a cause for concern.

Methicillin-resistant *Staphylococcus aureus*, also known as MRSA, is a unique strain of *S. aureus* that is resistant to several antibiotics; specifically methicillin, oxacillin, and penicillin with some strains now showing vancomycin resistance. The first methicillin-resistant *S. aureus* strain was identified in 1961. Since this first discovery, the prevalence of MRSA has grown substantially. MRSA is carried by about 2% of the general population, although many carriers do not show signs of infection. Previously, MRSA infections were reported to cause 1-5% of *S. aureus* infections in hospitals. Unfortunately, the spread of *S. aureus* and MRSA has not stopped at the healthcare setting. In recent years, the infection that originated in hospitals has also been reported in healthy individuals in the general public. Common symptoms of infection include boils, pustules, and skin rashes; however, severe infections can cause pneumonia, osteomyelitis, and bacteremia. MRSA is now considered a major public health concern because it is no longer isolated to healthcare facilities and is adapting to resist antibiotics. Many people are now carriers of *S. aureus* or MRSA, which puts them at an increased risk for developing an infection or spreading it to others.

Based on risk factors, infection type, and susceptibility patterns, MRSA infections can be divided into two groups, community-associated (CA-MRSA) and healthcare-associated (HA-MRSA) infections. HA-MRSA infections are typically found in patients in hospitals and long-term care facilities. Infections are also typically found in those who receive kidney dialysis treatments, cancer treatments, use illegal drugs through injection, or have had surgery in the past year. Symptoms of this type of infection tend to be severe and include infections of the blood, heart, lungs, and other organs, leading to fevers, chest pain, and fatigue. CA-MRSA infections account for 85% of all MRSA infections.

Although MRSA first emerged as a healthcare-associated infection, it has now spread to the community outside of healthcare settings. CA-MRSA occurs in healthy people who may have been exposed in close contact with others in schools, day cares, sports teams, and prisons. In addition, CA-MRSA can be spread by sharing clothing, towels, razors, and sporting equipment due to its ability to survive on fomites. Many people are able to fight off the infection and never experience any symptoms, however people who have compromised immune systems may be unable to fight it off and become infected.

Not only do HA-MRSA and CA-MRSA differ in how they are contracted, but they also have differences in antibiotic resistance profiles and average age of people infected. HA-MRSA patients are usually older than...
CA-MRSA patients. A study published by the Journal of Clinical Microbiology reported that the average age of a person with HA-MRSA was 54 while the average age of a person with CA-MRSA was only 39.14 In addition, HA-MRSA strains are resistant to a wider range of non-beta-lactam antimicrobials than CA-MRSA. A study by Sievert et al. (2012) noted that HA-MRSA infections were, “...more likely to be resistant to ciprofloxacin, clindamycin, gentamicin, levofloxacin...” Typically CA-MRSA is resistant to clindamycin whereas CA-MRSA is susceptible to clindamycin. 12 Therefore, susceptibility to antibiotics, along with other methods, has been described as an accurate method for determining whether an infectious strain is most likely HA- or CA-MRSA.

Healthcare professionals have been found to be common carriers of S. aureus. This poses a particular threat to the public given that patients are frequently immunocompromised or have open, post-operative wounds. Any healthcare personnel that are carriers of S. aureus could potentially spread the bacteria to a patient simply through contact with their hands. Intensive care units, neonatal intensive care units, and pediatric units are particular locations where transmission could present the largest threat to patients’ wellbeing. Some patients of these units are young children who are premature, underdeveloped, or have undergone invasive surgeries, leaving them particularly vulnerable to S. aureus or other nosocomial infections. In 2011, Aswani and Shukla reported that of 202 participants enrolled in their study, 49 (24%) tested positive for carrying S. aureus and of this sample two (1%) were positive for MRSA.1 Bharathidasan et al. (2011) collected 44 samples from the nasal passages of healthcare workers. From these, 39 (88%) were positive for staphylococci and two (5%) were determined to be MRSA.7

The increase in the number of healthcare workers carrying S. aureus and MRSA between these two studies is evidence that MRSA is an escalating threat to patients. This fact, along with the ease of transmission and potential lethality of an infection, warrant an argument for the screening of healthcare workers. If healthcare professionals test positive for S. aureus or MRSA, proper precautionary measures can be taken when encountering patients, especially those with compromised immune systems. With these infections on the rise, all healthcare professionals need to become more aware of proper hygiene procedures in order to reduce the spread of these dangerous bacteria.

Materials and Methods
Sample Collection
Sterile calcium alginate swabs were moistened with sterile 0.85% NaCl saline solution. Each student volunteer inserted a moistened swab into their right nasal passages and twisted five times. This procedure was repeated with the same swab in their left nostril. To maintain anonymity, each sample was coded with a six-digit numeric sequence that only the volunteer recorded. The students who screened positive for MRSA were notified using the six-digit digital system and encouraged to follow up with their medical provider.

Mannitol Salt Agar
Immediately upon sample collection from the nasal passages, the swabs were plated on mannitol salt agar, abbreviated MSA, (BD BBL, Sparks, MD) to isolate Staphylococcus aureus. The sample plates were then incubated at 37°C for 24 hours. A positive result on MSA is classified as fermentation causing the agar to turn yellow, indicating the strain is Staphylococcus aureus. Each sample was observed for growth and fermentation of mannitol, and the results were documented.

Gram-Staining
An isolated colony from each sample above was stained using the Gram-staining procedure, and all resulting morphological characteristics were documented. This procedure was used to verify that our samples were consistent with the characteristics of S. aureus. Due to the composition of their cell walls, gram-positive bacteria, such as S. aureus, are able to retain the dye used in gram-staining and turn a violet color.

Blood Agar
All of the samples that were positive fermenters of the mannitol were plated onto Trypticase Soy Agar II with 5% sheep blood (BD BBL, Sparks, MD). Cultures were incubated at 37°C for 24 hours. Each strain was evaluated for hemolytic ability in order to help determine virulence. Hemolysis patterns alone do not determine MRSA or distinguish between CA-MRSA and HA-MRSA; however, the ability to lyse red blood cells is considered to be a characteristic of pathogenic bacteria. Beta hemolysis is defined as the complete breakdown of red blood cells while oxygen-sensitive beta hemolysis is a complete breakdown of red blood cells in an anaerobic environment. Alpha hemolysis is the reduction of hemoglobin to methemoglobin in red blood cells without destroying the cell walls. Gamma hemolysis is characterized by no lysis of red blood cells.13

CHROMagar
Isolated strains from MSA plates were streak plated onto CHROMagar II plates (BD BBL, Sparks, MD). The plates were incubated at 37°C for 24 hours and results were interpreted and documented. The plates were scored positive for MRSA if the colonies were mauve-purple in color and negative for MRSA if the colonies were colorless. Blue growth was recorded as Methicillin-sensitive S. aureus (MSSA) as instructed by the manufacturer.

Antibiotic Resistance Testing
Overnight bacterial cultures were made for each strain using an isolated colony inoculated into 3mL LB broth. The overnight cultures were placed in an automatic shaker set at 110 revolutions per minute at 37°C for 24 hours. Using a micropipette, 100µL of each overnight broth culture was placed in a cuvette and tested using a spectrometer. Using 0.85% sterile NaCl solution, the OD600 of each solution was diluted to 0.12-0.14.

Each diluted solution was plated onto a Mueller-Hinton agar plate (BD BBL, Sparks, MD) using a sterile swab. The swabs were used to make three smear plates of each strain. On the first two plates for each strain, four antibiotic testing disks (BD BBL, Sparks, MD) were placed using sterile forceps. Plate A contained clindamycin (2mg), oxacillin (1mg), linezolid (30mg), and doxycycline (30mg). Plate B contained rifampin (5mg), vancomycin (5mg), vancomycin (30mg), and a sterile blank disk. The third plate labeled plate E contained and oxacillin E-test strip (Biomerieux, Durham, NC).

All of the plates were incubated at 37°C for 24 hours. The resistance to each antibiotic was determined by measuring the diameter of the growth inhibition around each disk and specifically interpreted based on manufacturer instructions for each drug. E-test strips are used to deliver a gradient of concentrations of oxacillin to surrounding bacteria. The resistance for the E-test strip was determined by the level at which growth ceased at the bottom of the ellipse, per manufacturer instructions. Bacterial growth at a concentration of 4.0μg/ mL or greater is classified as MRSA.

Long-term Storage of Samples
All strains were preserved in 50% sterile glycerol and Luria Bertani (LB) broth and stored at -80°C for long-term storage for future testing and reference.

Semester Trials
Two separate trials were performed, one in the spring of 2013 and another in the fall of 2013. All protocol remained the same throughout both trials.
Results

The aims of this study were to identify the prevalence of *S. aureus* and MRSA within a population of nursing students and determine if the strains found in this population had characteristics of HA- or CA-MRSA. The results of this study show a need for improved precautions and educational programs to be put into place to prevent the spread of infection.

Spring 2013

A total of 36 samples were collected from a population of students at St. John Fisher College enrolled in BIOL 107L Microbes and Disease. Our sample size was relatively small due to our limited availability of willing participants who were considered to be a part of the healthcare field. We obtained 11 samples (31%) that were positive fermenters on mannitol salt agar, indicative of *Staphylococcus aureus* (Figure 1). Individual strains were identified and separated for further testing. From the 11 samples, 19 different strains of *S. aureus* were isolated. Isolated colonies from these samples were determined to be Gram-positive cocci.

When 18 of the 19 strains were plated onto blood agar, there were 12 strains that were β hemolysins with complete hemolysis, five that were oxygen-sensitive β hemolysis, and one that was a γ hemolysin with no hemolysis (Table 1 and Figure 2). Only 18 of the 19 strains were used for further testing because one strain was unable to be reproduced and isolated again after a second round of MSA plating. These 18 strains were then tested for MRSA; 13 of them tested positive resulting in purple colonies, five tested negative and resulted in colorless growth (Table 1).

The 18 strains tested on CHROMagar were evaluated further for antibiotic resistance. A total of 15 strains were susceptible to all tested antibiotics: oxacillin, linezolid, doxycycline, rifampin, vancomycin, and clindamycin. Clindamycin was the only antibiotic that showed variation in resistance patterns. Strains 363477 and 508236B were completely resistant to clindamycin; growth was not affected and the zone of inhibition was 0mm (Table 1). Strain 363477 showed no hemolysis and was negative on CHROMagar. Strain 508236B demonstrated oxygen sensitive hemolysis and was positive on CHROMagar. Strain 334475B had intermediate resistance with a zone of inhibition of 15mm (Table 1). Strain 334475B showed beta complete hemolysis and was negative on CHROMagar. When subjected to an oxacillin E-test strip, all of the strains were susceptible.

### Table 1. Characteristics of strains classified as *Staphylococcus aureus*. β indicates beta hemolysis; β₀ indicates oxygen-sensitive hemolysis; γ indicates gamma hemolysis. R represents resistance, I represents intermediate resistance, and S represents susceptibility to an antibiotic.
After collecting 26 nasal samples from St. John Fisher College nursing students, 11 samples fermented mannitol (Figure 3). These 11 samples produced 13 different isolates, which were all determined to be gram-positive cocci. Our sample size was relatively small due to our limited availability of willing participants who were considered to be a part of the healthcare field. Following gram staining protocols, the isolates were dyed and visualized under a light microscope. All of the samples were stained purple, indicating gram-positive bacteria. They were described as being cocci that assembled into “web-like” structures, indicative of Staphylococci. Due to these characteristics, 50% of the total sample size was considered Staphylococcus aureus. Of the 13 isolates, 11 displayed β hemolysis, one displayed α hemolysis and one showed γ hemolysis when grown on blood agar (Figure 4).

Once the isolates were classified as S. aureus, additional testing was performed in order to determine antibiotic resistance profiles for each strain. We determined that six samples (46%) were intermediate or resistant to at least one of the six antibiotics tested (Table 2). Of the six strains, five demonstrated either intermediate resistance or resistance to clindamycin (Table 2). Strains 241591A, 170382A, 261312A, 349818A, 171819A, and 111216B were found to be susceptible to all drugs tested. After performing an E-test strip test, one isolate was defined as MRSA. In order to affirm this classification of MRSA, there had to be an area of clearance within the lawn of bacterial growth at 4.0 μg/mL or higher. As shown in Figure 5, strain 170382B had an E-test strip reading of 128 μg/mL, confirming it is indeed MRSA. Of the total sample size, 4% were considered MRSA. Finding 50% S. aureus and 4% MRSA from a population presents a major problem. The prevalence of natural colonization of S. aureus is clearly on the rise, based on previous statistics, and this virulent pathogen can cause many diseases within the human population.
Discussion

Based on the findings of this experiment, it is clear that *Staphylococcus aureus* is present in healthy individuals. From the 36 samples collected in spring of 2013, 11 of them were positive for *Staphylococcus aureus* (31%) but none were determined to be MRSA. This number is consistent with the statistic that about 30% of the population are carriers for *S. aureus*. We are not surprised that none of our strains were considered to be MRSA considering only 2% of the general population are carriers of MRSA. It was determined that *S. aureus* colonized 50% of our sample size in fall of 2013, and 4% were said to have MRSA. This statistic is greatly increased from the fall trial and from numbers previously published, declaring 0.2-7% MRSA. In previous studies conducted in 2011, 2% of the sample size were infected with MRSA. Only three years later, the number of people infected in this study had already increased to 4%. Our results are consistent with the increase in *S. aureus* becoming part of the normal flora.

An interesting contradiction arose when comparing CHROMagar II results with antibiotic resistance to oxacillin E-test strips. Of the 18 strains from the spring of 2013, 14 were positive for MRSA on CHROMagar; however, none of the 18 strains were positively identified as MRSA when subjected to antibiotic resistance testing. This brings about the question as to why these strains would produce a positive CHROMagar result, a test commonly used to classify MRSA, yet not technically be considered MRSA due to antibiotic resistance. At first we thought it might relate to the virulence of the bacteria. However, there was no common pattern when looking at the hemolysis patterns of the strains that tested positive on CHROMagar.

In addition, antibiotic resistance testing also showed no obvious pattern when compared to CHROMagar results. Unfortunately, CHROMagar II is patented, and we are unable to obtain information on exactly what the agar is testing for to classify bacteria as MRSA. We would like to suggest future research into the mechanism that CHROMagar is targeting in order to understand why these strains produce a false positive. Nonetheless, it is important to note that these strains, although not being MRSA, clearly show some common characteristic with the resistant bacteria if it is providing such readings on the CHROMagar. They could still have potentially harmful effects on humans. Since hemolysis is the ability of bacteria to lyse red blood cells and is therefore an indicator of virulence, it is important to note that there were naturally occurring strains of potentially harmful *S. aureus*. If these bacteria are resistant to common antibiotics they could also cause infections that people may struggle to eliminate from their bodies.

It was difficult to determine if the isolated strains were HA- or CA-MRSA based on our results. None of the strains from the spring of 2013 were considered to be MRSA and therefore would not qualify as MRSA. However, due to the fact HA-MRSA is commonly resistant to clindamycin and CA-MRSA is susceptible to a wider range of antibiotics, it is reasonable to suggest our isolated MRSA and *S. aureus* strains were more closely related to HA-MRSA. The small sample size could be a potential reason for the lack of clear patterns in the results obtained. It is possible that a larger sample size would allow for patterns among hemolysis, CHROMagar results, and antibiotic resistance to surface. However, one important theme that these results point to is the diverse variation in characteristics of bacteria, even in such a small population.

Regardless of the fact that only one of our collected samples was positive for MRSA, it is still noteworthy that some of the strains demonstrated both virulent properties and antibiotic resistance. These samples were collected from a population of students who plan to enter the medical field and will be in direct contact with patients. It is crucial that these students learn the proper techniques to prevent the spread of such microorganisms to patients, especially in a medical setting where immune systems may be compromised.

If *S. aureus* and MRSA infections are truly on the rise, then new techniques and policies may need to be put in place within areas such as hospitals and nursing homes in order to protect the patients. These bacteria can be spread easily from healthcare professional to patient and vice versa. This mode of transmission is very common due to the fact that *S. aureus* is often carried on one’s skin and can enter a host via any open wounds, mucosal membranes, skin lesions, and other similar sites. Individuals residing in hospitals, nursing homes, or other healthcare facilities are much more likely to have these open wounds and lesions that allow for easy transmission. With this in mind, all healthcare professionals may need to have prior screening for these bacteria before working with patients. If the healthcare worker were indeed colonized with such bacteria, they would be able to take the proper precautionary steps when working with patients. This method could prevent the spread of infection before it has the potential to harm the patient.

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References


