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Microbiology of Our Schools: Does Having a Wrestling Room Increase the Risk of Outbreaks of Methicillin Resistant *Staphylococcus aureus* in the Community?

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Abstract

Methicillin Resistant Staphylococcus aureus has been a very big cause of infection for many years. In recent times a new strain of this bacterium has emerged, known as Community Associated MRSA (CA-MRSA). In my experiment, I sampled different areas around a school including the kindergarten center, the high school, the varsity wrestling room, and the weight room. I used special agar plates known as HardyCHROM MRSA contact plates that isolated the bacteria, and then I performed DNA tests on the bacteria, known as polymerase chain reaction to see if the colonies were MRSA. I found three different colonies of a spore producing gram positive bacteria known as Bacillus in the weight room as well as another three samples of the same bacteria in the wrestling room. Also I found a different species of Staphylococcus known as *Staphylococcus haemolyticus* in the wrestling room. This shows that the bacteria are traveling from building to building and this could be a major problem when wrestling season comes around due to the increase in amount of bacteria in the wrestling room.

**Introduction**

The gram-positive bacteria MRSA, also known as Methicillin Resistant *Staphylococcus aureus* has been a large problem around the world for many years. It has gotten worse recently. The concern for MRSA in the world today is because of its high morbidity and mortality rate, as well as it being resistant to many antibiotics, making it even harder to exterminate.*1 Staphylococcus aureus* is the most dangerous species of the Staphylococcus genus, but with a resistance to many common and effective antibiotics, it becomes even more dangerous. MRSA is usually spread through direct contact and can cause severe problems such as infections and pneumonia6.

MRSA has been a problem in hospitals for a long time. When MRSA is associated with a healthcare setting such as a hospital or a nursing home, it is known as HA-MRSA or Hospital Associated Methicillin Resistant *Staphylococcus aureus*. About two in 100 people carry HA-MRSA6, the number of people getting an actual infection being much less than that, but still the number of people who carry it, considering how dangerous it is, is worrying.

MRSA has recently emerged as problem in the community as new strains known as CA-MRSA or Community Associated Methicillin Resistant *Staphylococcus aureus* have arisen. The difference between HA-MRSA and CA-MRSA is that CA-MRSA can affect anyone, has fewer resistances but it also reproduces quicker, and is usually a skin infection while HA-MRSA is usually in the blood stream and is restricted to hospitals. The big problem with CA-MRSA is that no one knows where it comes from, how it emerged, and what the reservoir is. One very informative paper spoke about environmental sampling in thirty-three Washington state fire stations for MRSA. They were able to isolate MRSA from 8.0% and MSSA (also known Methicillin Susceptible Staphylococcus aureus) from 18.5% of the 653 samples. These percentages suggest that in areas of high contact, MRSA and MSSA are much more prevalent.7

CA-MRSA has also recently been a big problem in the world of football and wrestling. One very public example occured last year when three Tampa Bay Buccaneers football players were diagnosed with MRSA.2 Another journal article spoke about how many different skin diseases occur frequently on the wrestling mat, such as impetigo and of course MRSA.3 A very worrying study showed that wrestlers are 16 times more likely to get MRSA during tournament season, a tournament being when you could wrestle up to 5 matches in one day with no showering in between.4 In a survey performed in Iowa by Pedersen and his colleagues, they found that 57% of high school athletic programs that responded to the survey had at least one athlete that acquired a soft tissue infection, 10.8% of those soft tissue infections were reported as MRSA.9 Another important study, done by Collins and her colleagues, found that a majority of outbreaks reported occurred in close contact sports such as wrestling and football. Twelve of these 21 outbreaks occurred at a high school or collegiate level.8 It is believed that the cause of MRSA outbreaks is the lack of equipment being cleaned properly.5

My project involved using HardyCHROM MRSA contact plates to attempt to collect MRSA from several high population areas in the wrestling room, weight room, and from around a school. This was done during the summer. The weight room was still in use because of open gym and football workouts, as well as the school still being in use because of summer school and driver’s education. Unfortunately the wrestling room was not in use during this time and will not be in use until later November when wrestling season starts. This study was conducted to show how the wrestling room and weight room might be a source of CA-MRSA, as well as to show how bacteria such as MRSA or Bacillus could travel easily from place to place.

**Materials and Methods**

In this study, samples were taken from the high school itself, the weight room in the high school, and the wrestling room across the street used by the varsity wrestling team. Sampling was performed using HardyCHROM MRSA contact plates with the procedure shown below. Then, colonies that looked as if they were Staphylococci, were streaked those on to an agar plate. DNA was isolated and PCR was performed. Following that BLAST analysis was performed to identify the DNA sequences.

Materials:

HardyCHROM agar (Hardy Diagnostics) and nutrient agar (BD), EASY DNA Kit (Life Technologies), Choice Taq Mastermix (Denville Scientific Inc).

Procedure for sampling:

First I obtained 32 plates for the wrestling room, school and weight room, used 10 on various areas in the wrestling room, as well as 10 in the weight room, and 12 for the school. Then plates were pressed over the area of desired surface for 10 seconds. Plates were incubated at an inverted position for 18 - 24 hours at 32°C. Plates were examined for colonies showing typical morphology and color. Then I streaked presumptive staphylococcal colonies onto nutrient agar.

Procedure for DNA extraction:

First I took a portion of bacteria off an overnight plate and added to it to 300 ml of Tissue cell lysis and was it vortexed completely. Then in separate tubes I added silicon beads to a tube until they reached above the bevel at the bottom of the tube. Following that I lysed the bacteria with the bead beater- speed 6.0 for 30 seconds; until the tubes were full of foam. Then I centrifuged tubes for 10 minutes at 16000 and 4 degrees Celsius (foam settled). Next I removed supernatant and placed it in a 1.5 microliter Eppendorf tube, and then I discarded the pellet. Following that I added 150 microliters of the MPC protein precipitate reagent. Then I vortexed for 10 seconds, until the liquid became cloudy. Next, I centrifuged the tubes for 10 minutes at 16000 and 4 degrees Celsius. Then I transferred the Supernatant to a new 1.5-microliter Eppendorf tube and discarded the pellet. Next step 500 microliters isopropanol was added to each tube and invert several times to mix. Then I centrifuged the tubes for 10 minutes at 16000 and 4 degrees Celsius and discarded Supernatant. Then I washed the pellet twice with 75% ethanol. After removing most of the ethanol, I used the quick spin technique on tubes to collect remaining ethanol at bottom of the tube. A 10 microliter pipette was used to remove the last bit of the ethanol. Then I air dried pellets for 10 minutes at room temperature. Next, I re-suspended pellet in 50 microliters of H20. If the pellet was invisible add the H20, vortex thoroughly, let stand a few minutes and vortex again. DNA should be stored at -20 Celsius.

Procedure for PCR:

First I added 2 microliters of DNA, 4 microliters of Primer Forward, 4 microliters of Primer Reverse, 15 microliters of dH20, and 25 microliters of Denville mix. Then the samples were put in the PCR machine for 3 hours for 31 cycles. Finally sequencing was performed by Macrogen.

**Discussion of Results**

 The proper cleaning of equipment can prevent MRSA very easily along with the proper hygiene as instructed by the CDC.11 But the big problem is that a lot of schools don’t go through these procedures well enough, and that is how people end up getting a MRSA infection. I sampled three different places in total, around the halls of the school, the weight room, and the varsity wrestling room. For my first round of sampling, I took twelve samples in the high school, ten in the weight room which is located in the high school, ten samples in the kindergarten center, and ten in the wrestling room which is located in the kindergarten center. Out of these samples, I found two different types of Bacillus but two of the same type on two different agar plates. So in total, three of the plates had Bacillus. In the high school and kindergarten center, no bacteria grew on the plate. While in the wrestling room I found three more samples of bacillus, the same species as the bacillus that was found in the weight room. Also, in the wrestling room, I found one sample of *Staphylococcus haemolyticus*. The reason there was such a lack of bacteria in these two school areas is due to the lack of contact in a school environment as compared to the wrestling room and the weight room. Also, another factor that could have contributed to this was the fact that the contact plates that were used in this project are specialized for picking up MRSA and other gram positive bacteria such as Bacillus. I found three different samples of *Bacillus thuringiensis* in three different places in the weight room, two different samples of *Bacillus thuringiensis* in two different places in the wrestling room, and one sample of *Staphylococcus haemolyticus*. So overall, there were zero colonies of bacteria found in the high school and the kindergarten center, while three samples of *Bacillus thuringiensis* were found in both the wrestling room and the weight room, and one sample of *Staphylococcus haemolyticus* was found in the wrestling room. I created a flow chart showing the foot traffic around the three main schools in the district that were suspected for MRSA.

Figure 1- This picture shows the layout of the schools that the bacillus found could move to, as well as the layout of the school system.

I believe that the Bacillus found in the weight room and wrestling room came from the High School field because the species of *Bacillus thuringiensis* is found in pesticides, which are used on the field next to the highschool.10 This shows how easily any bacteria can move around considering it got all the way across the street onto the currently unused wrestling mats. I suspect that the janitors are carrying these bacteria from building to building. Also, this means that some of the bacteria are most likely on the turf field at the elementary school as well as in the football locker room, both areas have high amounts of contact. Below are three pictures of sampling in various places.

Figure 2, 3, and 4 all show me sampling various areas around the schools with the correct technique for the HardyCHROM agar plates.

Figure 2- This picture shows me sampling a piece of equipment in the weight room with one of the HardyCHROM agar plates.

Figure 3- This picture shows me sampling a door handle in the Kindergarten center with one of the plates.

Figure 4- This picture shows me sampling one of the dumbbells in the weight room with a plate.

**Conclusion**

The experiment so far, has helped show how easily bacteria can move from one area of high contact to another area of high contact. Sampling shall be performed again in November once wrestling season starts to see if MRSA can be isolated. Results from the first round of sampling to the second round of sampling will determine if the wrestling season affects the amount of bacteria in the wrestling room. I predict that there will be more bacteria during wrestling season because of the increased amount of contact, the increased temperature, and the increased use of the room. The reason that it will be a higher temperature in the room is because the room is heated during practices to help wrestlers lose weight. Most times during the teams practice, from 3:30 PM to around 5:30 PM, the room is heated to around 80 degrees Fahrenheit. One major improvement I could have made to my project was to sample the football locker room at the elementary school, because if Bacillus would have been found, it would have showed how the bacteria is moving to all of the areas of high contact. While more trials have to be performed when wrestling season starts, the fact that Bacillus was found in different buildings show how easily bacteria can travel. Also the *Staphylococcus haemolyticus* found in the wrestling room shows that even in the offseason, the mats could host many different forms of bacteria. In conclusion, something needs to be done about the ease of bacteria traveling, such as, mats on the ground outside the school buildings that have a solution on them that will kill the bacteria on the person’s shoes. This is very possible because it is now a requirement to have a mat that wrestlers step on before their match that has a cleaning solution on it so they do not spread bacteria from their shoes on to the mat. If we were to put these mats outside of the school buildings, this would help keep areas like the weight room and the wrestling room much more bacteria free, this would be important to do soon because once wrestling season comes around and the amount of bacteria traveling increases, it could be detrimental to someone’s health, as it was for one of my close friends. But all of this could be avoided if the CDC’s guidelines for the prevention of MRSA were followed and the wrestling room was kept cleaner.

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