Viability of Methicillin-Resistant *Staphylococcus aureus* on Artificial Turf Under Outdoor and Laboratory Environmental Conditions

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This thesis titled

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ABSTRACT

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Viability of Methicillin-Resistant *Staphylococcus Aureus* on Artificial Turf Under Outdoor and Laboratory Environmental Conditions

Director of Thesis: Andrew Krause

Methicillin-resistant *Staphylococcus aureus* has survived on artificial turf in a laboratory setting when provided a nutrient source. There is limited evidence on the viability of MRSA in outdoor environmental conditions. This study compared the survival of MRSA in a laboratory environment to an outdoor environment over seven days. Artificial turf was inoculated with MRSA strain USA300 and exposed to laboratory and outdoor environmental settings. Samples were collected daily. MRSA survival was determined by growth on CHROMagar plates. Results indicated a difference in the mean survival time of MRSA between a laboratory environment (7.00 ± 0.00 days) and an outdoor environment (4.67 ± 2.52). Conditions including surface temperature, ambient temperature, relative humidity, precipitation and solar radiation may have affected MRSA survival. Future research should explore the effect of specific environmental conditions on MRSA survival and the effect of nutrients on outdoor survival.

Approved:_____

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CHAPTER 1: INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of the *Staphylococcus aureus* bacteria that has become resistant to beta-lactum antibiotics such as methicillin, penicillin, and oxacillin.[1-4] MRSA has been identified as a public health risk by the Centers for Disease Prevention and Control.[1] MRSA often infects otherwise healthy people and can be spread through person-to-person contact or person-to-surface contact.[2, 5, 6] MRSA commonly presents as a painful boil or pimple. Complaints of unusually severe pain and soft tissue necrosis are associated with the Paten-Valentine leukocidin exotoxin, a property of the bacteria.[6-8] Unreported infections can morph into severe cases in a short amount of time.[9] The USA300 MRSA strain has been confirmed in cases resulting in necrotizing fasciitis, pneumonia, and death.[6-8] The USA300 strain is the most common in the USA and has been found in athletes.[7, 10, 11] Bacteria is the second most common cause of skin infection in athletes, with football reporting the most MRSA exposures.[12,13]

MRSA has become increasingly problematic within athletic settings and have been well documented.[3, 6, 7, 11, 13-30] Athletes are at higher risk of obtaining MRSA infections, with contact sports reporting more incidences of infection versus non-contact sports.[6, 18, 31] Athletes encounter risk factors such as skin-to-skin physical contact, contact with contaminated surfaces, insufficient hygiene of athletic equipment and clothing, inadequate care of skin lesions, and broken skin caused or exacerbated by turf burns.[1-3, 6, 12, 13, 15, 17-21, 27, 29, 31, 32] MRSA infections occur within high school, club, collegiate, and

professional sports.[25, 29, 32-34] Patients hospitalized due to infection are most commonly football players.[31]

MRSA infections affect multiple facets of an athlete's life. Team activity is interrupted as the participant must be removed from sport for up to 10 days with possible hospitalization.[12, 33] Infection has an opportunity to spread among teammates prior to removal from sport due to environmental and person-to-person spread of bacteria.[35] Specific antibiotics are required to treat the infection with associated higher cost.[1-5, 19, 35]

A potential mitigating risk is participation on artificial turf. Installation of artificial turf has increased among high school, collegiate, and professional institutions. Athletes participating in contact sports on artificial turf have an increased likelihood of developing skin abrasions, such as turf burn, which can provide entry to pathogens.[16] According to the American Academy of Dermatology, football reports the most MRSA exposures to athletes, with turf burns increasing their chance of infection.[13] Evidence has also shown athletes with turf burns have higher infection rates than those without.[18]

MRSA is capable of surviving indefinitely when grown in a laboratory, provided nutrients, and placed on artificial turf.[36] MRSA thrives at 25°C and 11-33% relative humidity; higher humidity decreases MRSA survival.[12,18] MRSA can be spread via contact with surfaces containing bodily fluids.[23] Saliva and nasal secretions contain mucin, which can be a nutrient source for MRSA.[13, 17] These nutrients can be deposited on artificial turf fields used by athletes.[17, 36] Removal of the nutrient source limits survival of MRSA to three days in a controlled climate.[36]

In situ evidence of MRSA in athletic facilities exists, including weight rooms, athletic training facilities, wrestling facilities, and locker rooms.[25, 32] Sanitation of these facilities can be achieved through proper application of solvents and has been suggested by position statements.[1, 37] Maintenance of athletic fields is missing in the discussion of MRSA infection management. The ability of MRSA to survive on artificial turf in an on-field, outdoor environmental setting is unknown. Decreased MRSA presence, caused by exposure to outdoor environmental conditions, would establish a window in which the risk of contamination due to artificial turf would be reduced.

The focus of this thesis is to determine the viability of MRSA on artificial turf when exposed to outdoor environmental and laboratory conditions.

Statement of the Problem

Research has established the viability of MRSA in controlled climate conditions with and without a nutrient base; however, evidence does not exist concerning the ability of MRSA to survive on artificial turf surfaces in an outdoor environment. The effect of environmental conditions on MRSA survival has not been demonstrated, specifically ambient temperature, surface temperature, humidity, precipitation, and solar radiation.

Purpose

The purpose of this thesis was to examine the survival of MRSA on artificial turf in an outdoor environment and a laboratory environment over a 7 day period. The primary aim of this thesis was to identify the period of time MRSA is capable of surviving on artificial turf in an outdoor environment and to record the environmental

conditions (ambient temperature, surface temperature, humidity, precipitation, and solar radiation) occurring during the sampling period.

Significance of the Study

The objective of this thesis was to determine the ability of MRSA to survive over a specific time period while exposed to environmental conditions similar to those found annually in athletic settings. This thesis examined a variety of variables including MRSA survival on artificial turf without additional nutrient supply, surface temperature, ambient temperature, humidity, precipitation, and solar radiation. This specific combination of variables has not been observed in prior literature. Measurement of variables affecting the viability of MRSA provided data relevant to artificial turf in the athletic setting and the environmental conditions capable of naturally reducing the risk of MRSA infection.

Research Question

The research question guiding this thesis was:

1. Are there differences in the viability of MRSA on artificial turf between laboratory conditions and outdoor environmental conditions?
   Environmental conditions (surface temperature, ambient temperature, relative humidity, precipitation and solar radiation) were recorded during the sampling period and are presented in the results and discussion.

Null Hypothesis

H_{01}: There is no difference between the viability of MRSA on artificial turf in a laboratory setting and an outdoor environment.

Delimitations of the Study

1. This study was conducted on unused artificial turf without additional application of surface disinfectant. A sample of the turf was obtained by swab and applied to a CHROMagar plate prior to experimentation to establish a negative control.
2. This study was conducted in a natural environment with exposure to weather conditions occurring in Athens, OH during the months of March and April, 2012.
3. The artificial turf surface was not exposed to typical human physical activity. No potential athletic nutrient base (ie nasal secretions and saliva) were present.

Limitations of the Study

This study was conducted with the following limitations:

1. This research was collected in Athens, OH in March and April, 2012. Environmental conditions occurring during the study are limited to those naturally occurring at the site and may be difficult to replicate.
2. Environmental conditions were measured at the time of sample collection. The conditions were not continually recorded but were measured every 24 hours during the trial periods.

Definition of Terms

Artificial turf. A synthetic surface with a grass-like appearance. Artificial turf is commonly installed at athletic facilities as a playing surface for multisport use.

Outdoor environmental conditions. Weather conditions occurring during the months of March and April, 2012. These conditions were not controllable. Conditions were measured by SCALIA lab and recorded daily from their website.

Community acquired Methicillin-resistant Staphylococcus aureus (CA-MRSA). A mutation of the MRSA bacteria which commonly causes skin infections due to the Panton-Valentine leukocidin produced by genes in its DNA,[1, 8, 10] affecting people without predisposing risk factors.[3, 6, 7]

Hospital acquired Methicillin-resistant Staphylococcus aureus (HA-MRSA). A mutation of the MRSA bacteria seen in highly populated healthcare settings and associated with risk factors such as recent hospitalization.[7, 16]

Methicillin-resistant Staphylococcus aureus (MRSA). A strain of the *Staphylococcus aureus* bacteria that has become resistant to beta-lactum antibiotics such as methicillin, penicillin, and oxacillin.[11]

Viability. The survival over time of bacteria.

CHAPTER 2: REVIEW OF LITERATURE

Methicillin-resistant *Staphylococcus aureus* has become a global problem since it was identified in 1961 and is now one of the most studied antibiotic-resistant bacteria.[3] This bacteria is the most common cause of cutaneous infections in emergency rooms in the United States.[38] The CDC has identified MRSA as a public health risk, with up to 30% of the population carrying the bacteria nasally.[1, 23] The following review will provide background concerning the history, signature of infection, laboratory survival, prevalence in the healthcare community and in athletic populations, and artificial turf's role in the risk of MRSA infections.

History and Bacteriological Design of MRSA

Methicillin-resistant *staphylococcus aureus* is a strain of the *Staphylococcus aureus* bacterium containing the mecA gene, a penicillin-binding protein causing resistance to β-lactum antibiotics such as erythromiacin, oxacillin, methicillin, and penicillin.[2, 3, 19, 26, 37, 39, 40] Bacteria survivability is dependent upon its resistance genes.[41] Increased use of methicillin in the 1960s was followed by the emergence of resistant strains in 1961.[6, 20, 39, 42] Microscopically, MRSA is a round, clustered gram positive bacteria surviving via aerobic and anaerobic respiration.[28, 39]

Variations of the MRSA Bacterium

Staphylococcus aureus bacteria can be divided into 2 groups based upon their reaction to β-lactum antibiotics: methicillin-resistant (MRSA) and methicillin-susceptible (MSSA).[9] Genetic makeup and location of acquisition divides MRSA into 2 groups: hospital-acquired (HA-MRSA) and community-acquired (CA-MRSA).[7]

Hospital-Acquired MRSA

Initially, the risk of acquiring MRSA in hospital settings outweighed the potential for contracting the bacterium in the community.[16] HA-MRSA can have SCC mecA type I or II chromosome cassettes, genes which differ from previous strands of the bacteria, making it more resistant to antibiotics than CA-MRSA.[7, 45] Treatment for patients with HA-MRSA can require operative care, increased risk of post operative infection, and extended treatment times.[43] Strands of HA-MRSA strands are commonly detected in nursing homes and high traffic health care facilities in addition to hospitals.[44] The MRSA bacteria have been known to be transported from the hospital setting to the community.[24]

Community-Acquired MRSA

CA-MRSA has the SCC mecA type IVa cassette, which produces Panton-Valentine leukocidin (PVL) toxin.[1, 8, 10] This is a neutrophil-destroying toxin that causes skin breakdown and infections and is present in 95% of CA-MRSA cultures.[6, 7, 29] The toxin has not been proven to effect the viability of CA-MRSA.[10] For this reason, CA-MRSA, specifically the USA300 and USA400 clones, are frequently found in the skin and soft tissue.[3, 10] USA300 is the most common MRSA strain in the United States and commonly causes reoccurring infections.[11, 43] This bacteria commonly affects members of the community who have no predisposing risk factors for contraction of the bacterium such as high school athletic teams.[44] Timely identification of a cutaneous infection caused by MRSA can reduce the risks associated with the bacteria as well as prevent transmission.

Signature of Infection

Methicillin-resistant *Staphylococcus aureus* frequently presents as a small, painful boil, lesion, or pimple that is reminiscent of a spider bite with a red ring encircling the abcess.[6, 18, 29, 45] Pain associated with the site is abnormally high considering the appearance of the lesion.[9] Infection commonly occurs in areas of decreased skin integrity.[17, 29] CA-MRSA can present as skin or soft tissue abnormalities such as abscesses, cellulitis, furuncles, carbuncles, folliculitis, impetigo, or paronychia in 75% of all cases.[9, 26, 46] Early detection of the infection may prevent later hospitalization. Fatal disease may occur due to MRSA infections that are not immediately detected.[9, 44] Necrotizing fasciitis, pneumonia, and death have been linked to the USA300 and USA400 strains.[7, 10, 29] Presence of fever and flu-like symptoms in conjunction with soft tissue adhesion warrant referral to a physician, in which case drainage, packing, and antibiotics are options for treatment.[29]

Treatment of MRSA

The gold standard for MRSA treatment is incision and drainage, coupled with antibiotic regimen.[1 ,2, 47] Lesions should be covered and kept clean.[47] Antibiotic treatment can last 7 to 14 days depending on severity and reaction to medication.[6, 47] Antibiotics frequently used to treat MRSA include clindamycin, tetracycline, intravenous vancomycin, fluoroquinlones, macrolides, and teicopanin.[1,39,48] Fluoroquinlones, macrolides, teicopanin, and vancomycin use is being discontinued due to increasing likelihood of resistance.[1,39]

MRSA Survival in the Environment

The most favorable condition for MRSA survival is 25°C with 11-33% relative humidity.[5] Higher relative humidity provides a less ideal environment, leading to less MRSA survival.[34] Ultraviolet light also decreases MRSA survival.[5, 23] Biofilms are more difficult to destroy via naturally occurring elements and antimicrobial products.[5, 49] Copper alloys have been used to decrease survival of bacteria in hospitals and have a detrimental effect on MRSA.[5, 50] Bacteria may be protected from dehydration in crevices on surfaces.[34] Bacteria deposited via blood, pus, or saliva are protected from the elements and are capable of surviving for longer periods of time.[34] Bacteria can be transferred from one environment to another by various modes of transportation, most commonly human carriers.[5]

Environmental and direct human contact largely contributes to the spread of MRSA. Humans may deposit MRSA on any surface they contact.[5] The nares, knuckles, forearms, fingertips, and shoes commonly carry bacteria colonies.[5, 26] One third of the population of the United States are colonized with MRSA in the nares.[51] Speaking and coughing, without secretion or phlegm, does not cause contamination; however, bacteria may exist in the air on particles relative to the size of skin cells.[51] Bacteria on the hands may be transmitted from surface to surface or area to area depending upon the original contamination surface.[16] More MRSA bacteria are usually present on floors, with up to 24,000 colony-forming units per square meter, increasing the risk of sole of shoe transmission from area to area.[5, 29]

MRSA bacteria are capable of surviving on multiple surfaces for extended amounts of time, allowing for increased transmission time. Dry or wet clothing, multipurpose surfaces, dry mops, sterile goods packaging, skin, and shoes all serve as a mode of transport for the bacteria.[5]

Decontamination Methods

Disinfectants are commonly used for prevention of MRSA in healthcare facilities. The reliability of cleaners used for hard surfaces to destroy MRSA colonies is dependent upon the concentration of the solvent, humidity, temperature, and exposure time on the surface.[5] Some MRSA strains also have biocide resistant genes, although resistance to disinfectants may not develop concurrently with a resistance to antibiotics.[5] Bleach, iodine, benzalkonium, and alcohol are effective in killing the MRSA bacteria on surfaces.[5] Cleaning solutions must also be properly prepared and used more than once per day, especially before and after high traffic occurs.[46] Proper techniques including mopping and spraying equipment and floors additionally helps control bacteria.[26]

Identifying MRSA

Proper specimen collection and incubation is essential when verifying presence of MRSA on testing sites. In order to collect a specimen in the most advantageous manner, varying methods of bacterial collection may be implemented depending upon the environment and the surface area available. This decision may be made depending upon the surface area being sampled. Nasal swabs may be taken to determine the colonization status of individuals.[19] Saline wash methods can be used to collect bacteria.[5] Enrichment broth may also be used during collection when incubation periods are intended to be

extended past the common 24 to 48 hours.[51] Brain heart infusion (BHI) broths and/or agars and sheep blood agars are commonly used in conjunction with MRSA studies.[5, 39, 45] In the event that a sample must travel a distance or over time prior to being cultured, an enrichment broth may also ensure that the bacteria is provided with enough nutrients to survive until plating.[20] In cases where swabbing is not used, additional methods of preparation may be completed to ensure a stable collection. Impression cultures of solid, small areas may be taken by pressing the surface directly to agar plates.[51] Brain heart infusion agar, sheep blood agar, and other commercial agar plates are available.[5, 39, 45] Cultures should be incubated at 35°C for 24-96 hours depending on the growth medium.[34]

CHROMagar Plates

Additional steps are taken to ensure that specimens are cultured in optimal conditions and correctly identified. CHROMagar plates are specialized agar plates which culture MRSA bacteria in 24 hours, quickly identifying the bacteria.[20, 39, 51, 52] The plates contain chromopeptone, sodium chloride, agar, a proprietary chromogen mix, and antifungal and antibacterial inhibitory agents which prevent the growth of additional bacteria during culturing.[20, 53] Previous studies have shown CHROMagar producing 95% of the MRSA bacteria, further emphasizing its 99.7% specificity.[20, 51] CHROMagar plates with bacterial samples are incubated in a dark incubator at 35°C to 37°C.[20, 51, 53] Mauve-colored colonies presented on the agar are considered a positive MRSA sample.[53] In order to determine the presence of MRSA, gram staining and coagulase testing may be

used, but are not required.[20, 51] Determination of the MRSA bacteria provides significant insight as to which antibiotics will be applicable in treating the infection.

Prevalence in the Community

Methicillin-resistant *Staphylococcus aureus* has become increasingly common in the community, causing risk for the general population and athletes. CA-MRSA infections occur in individuals who have fewer health risks common for infection.[5] Outbreaks in the community have not been limited to rural or urban settings.[54] Mass infections typically occur within one geographical area and affect the corresponding population.[54] Athletes are becoming a population at increased risk for MRSA infections. Athletes participating in volleyball, football, fencing, rugby, and wrestling continue to have documented cases of MRSA without instances of associated health risks.[16]

Prevalence in Athletes

Infection outbreaks are on the rise amongst athletic teams, with multiple cases reported among high school, collegiate, club, and professional sports.[2, 14, 15, 19, 21, 22, 25, 28, 29, 55, 56] Contact sports participants are at the highest risk of infection with football, wrestling, and rugby reporting the most infections.[2, 13, 18, 47] Often there are no infections reported within the athletic training, coaching, or other support staff of these teams.[17]

Skin abrasions are the most common athletic injury.[33] Abrasions commonly occur in areas left uncovered by sports equipment. Methicillin-resistant *Staphylococcus aureus* infections are also common in these areas, because breaks in skin integrity allow bacteria to infect the site.[19, 27, 28] These infections are most commonly found on the elbow, wrist,

chin, thigh, hip, forearm, knee, and tibial tuberocity.[17, 20, 25, 29] These infections are the second most common form of infection in athletes.[12]

Cost to Athletics

Athletes pay a *high price* when they become infected with MRSA. The individual loses practice and game time, and the team is at a higher risk of transmitting the bacteria amongst themselves.[33, 35] Athletes are typically held from participation for 72 hours following the administration of antibiotics. Some cases require athletes to sit out for 10 days.[12] Disinfection of commonly shared equipment is even more vital to reduce the risk of transmission.[6] Athletes occasionally miss school in order to seek treatment.[23] The cost of treating MRSA is increasing while the antibiotics available to treat the bacteria remain limited.[5, 20, 23]

Risk Factors and Athletics

Multiple factors associated with contact and noncontact sports causes increased risk for MRSA infection.[2] Repetitive skin-on-skin physical contact, specifically contact with a current infection or drainage site, can lead to transmission of bacteria.[2, 9, 12, 27] Breaks in the integrity of the skin via injuries occurring on the playing field such as abrasions, turf burns, and cuts, as well as body shaving, increase risk of infection.[6, 9, 12, 13, 15, 17, 18, 21, 28, 29, 57] Frequent, extended one-on-one exposure to infected individuals increases the rate of transmission.[9] Football linemen, cornerbacks, and wide receivers as well as athletes with a higher BMI have a greater risk if infection due to increased one-on-one contact with other players.[2, 9, 17, 25, 26] Sharing of personal hygiene items, clothing,

sports equipment, towels, lubricants, balms, and soaps lead to increased risk of infection.[15, 16] Frequent antibiotic usage can also predispose athletes to infection.[21]

Turf Abrasions

Turf abrasions, known as turf burns, have become an important risk factor for MRSA infection. Artificial turf is commonly found in athletic facilities and viewed by athletes as a equivalent to natural grass; athletes will slide across it effortlessly and without question, causing skin trauma.[3, 23] Turf lesions frequently occur in areas left uncovered by athletic equipment and can aggravate abrasions already occurring in these sites.[20] Bacteria can easily enter these large, damaged areas and cause infection.[2, 15] Athletes with turf burns have a 7 times greater risk of infection.[15] Position players who are already at a disadvantage have an increased risk of infection if they have turf burns.[17] MRSA can be transmitted through bodily fluids which have been secreted onto the artificial turf, such as saliva, nasal secretions, and blood.[23] Data concerning how long MRSA can survive on artificial turf using bodily fluids as nutrients are needed.

Artificial Turf and MRSA

The role of artificial turf in MRSA infections among athletics has come into question. Artificial turf surfaces have become increasingly popular because they require less maintenance and repair in comparison to their natural grass counterparts.[58] That less maintenance is required may cause concern when considering MRSA contamination. These fields may not be properly sanitized, increasing the likelihood of MRSA presence.

Survivability of MRSA upon athletic fields is also questionable. Research has demonstrated that MRSA is able to survive less than 3 days without a form of nutrition

on synthetic turf in optimal conditions (37^0C).[36] When provided with a nutrient source, MRSA may survive for weeks at optimal conditions.[36, 59] A viable nutrient source for the bacteria may be nasal secretions from athletes which are deposited on the turf surface, increasing the opportunity for contamination of an open wound.[36] Temperatures outside optimal conditions and environmental conditions such as relative humidity could decrease the survival rate of the bacteria.

Conclusion

Awareness concerning the importance of maintaining proper sanitation habits following abrasion on a playing field is vital in order to decrease the number of MRSA cases in the athletic population. Research providing information concerning the survival of MRSA on artificial turf surfaces provides better opportunities for athletic facility management and prevention of the spread of bacteria. Data could also provide sanitation information so that maintenance can be performed if environmental conditions have not exceeded optimal conditions for MRSA survival. Research using a controlled laboratory approach is needed to supplement our knowledge of MRSA.

CHAPTER 3: METHODS

This thesis was focused on determining if MRSA viability on artificial turf is altered by presence in an outdoor environment versus a laboratory environment. Environmental conditions occurring during sampling times were monitored and a significant effect on viability was hypothesized. This study addressed one research question:

1. Are there differences in the viability of MRSA on artificial turf between laboratory conditions and outdoor environmental conditions?

Design

We used a controlled laboratory study. The dependent variable was the presence of MRSA following exposure to environmental conditions (MRSA present or not present). The independent variables were time and environmental conditions. An inoculated plot and a control plot were placed in both test environments: an outdoor environment and a laboratory environment.

Population

The Ohio University Institutional Biosafety Committee reviewed and approved this study.

Instrumentation

Several instruments were used for data collection.

Methicillin-resistant *Staphylococcus aureus* strain USA300 (HealthLink, Inc. Jacksonville, FL, USA) was used because it commonly causes infections in an athletic population. Brain heart infusion enrichment broth (Bectin, Dickenson & Company,

Sparks, MD, USA) is a liquid nutrient source for MRSA. The bacteria are added to the sterile broth 48 hours prior to inoculation of the artificial turf to ensure the MRSA survives initial application. The broth is MRSA's mode of transport onto the artificial turf and the source of nutrition for the bacteria. Brain heart infusion (BHI) agar slants (Bectin, Dickenson & Company, Sparks, MD, USA) provide a nutrient source for MRSA, allowing the bacteria to survive prior to application on the artificial turf. CHROMagar MRSA II plates (Bectin, Dickenson & Company, Sparks, MD, USA) were used in this study due to a rapid response time and high specificity for USA 300 MRSA. Mauve-colored colonies are easily recognized on the agar surface. The MRSA bacteria, BHI agar slants and CHROMagar plates were refrigerated between 8°C-10°C prior to experimentation.

Phosphate buffered saline (PBS) is a solution used to rinse surviving MRSA off of the artificial turf during sampling. The saline was also used to store sterile forceps on the work surface before use. Sterile inoculating loops (Bectin, Dickenson & Company, Sparks, MD, USA) were used to transfer MRSA from the BHI agar slant to the BHI enrichment broth.

An autoclave (Consolidated Stills & Sterilizers, Boston, MA, USA) was used to sterilize the PBS and BHI enrichment broth. The autoclave uses a combination of heat and pressure to sterilize fluids. An incubator (Sheldon Manufacturing Inc., Cornelius, OR, USA) was used to maintain a constant ambient temperature for MRSA growth prior to inoculation and for proper use of CHROMagar plates for MRSA identification. The incubator was maintained at 35°C during experimentation.[36] An ExTech Mini IR hand-

held thermometer (Waltham, MA, USA) captured surface temperatures of the target collection areas using an infrared laser to pin point the exact data collection point.

The SCALIA Environmental Laboratory (Ohio University, Athens, OH USA) was accessed on line (http://www.scalialab.com/clip_conditions.html) to measure daily environmental conditions.

Data Collection Procedures

Establishment of Negative Control for Artificial Turf

A 7.62 x 7.62-cm swath of artificial turf was cut using a box knife. The blade was sterilized using isopropyl alcohol prior to use. Using sterile tongs, the swath was placed in a 500mL glass beaker. 20mL of PBS was poured over the swath, allowing the run-off saline to be collected in the beaker. A sterile swab was dipped into the runoff in the beaker and then applied to a CHROMagar plate. The swab was rotated during application to ensure optimal contact with the agar. The plate was incubated at 35°C and observed for mauve-colored colonies at 24 and 48 hours. No mauve-colored colonies were present within 48 hours and the artificial turf was used for the experiment.

Preparation of Artificial Turf Boxes

A 40.64 x 40.64-cm square of artificial turf was cut using a box knife and a cardboard pattern. A 30.48 x 30.48-cm square composed of 16 7.62 x 7.62-cm swaths was cut in the middle of the artificial turf. Each swath was numbered 1-16 on a diagram for identification when sampling. The artificial turf was placed in a 40.64 x 40.64-cm wooden plot. The upper right and left corners of each plot was labeled. Four plots with pre-cut artificial turf were created for each trial.

Figure 1. Outdoor environment plots with extracted swaths.

MRSA Agar Slant Preparation

The MRSA culture arrived on in individually wrapped swabs. Packages were stored in the refrigerator until they were prepared for application in the agar slant. The swab was allowed to reach room temperature prior to opening. When ready for inoculation, a package was opened and the swab removed. The fluid in the top of the swab was mixed with the solid at the bottom of the swab, releasing the bacteria into the fluid and onto the applicator in the swab. The applicator was applied to a BHI slant and placed in the incubator for 24 hours.

MRSA Broth Preparation

In order to inoculate the artificial turf, the MRSA isolate was grown in a nutrient broth. BHI nutrient broth was prepared by adding 37g of the medium to 1000mL of boiling distilled water in a 1000mL beaker. The beaker was heated with constant agitation using a heating plate with a stirring setting, allowing a magnetic stirring rod to spin in the bottom of the beaker. The broth boiled for 1 minute. The broth was then poured into a flask, and the mouth of the flask was covered with aluminum foil and autoclaved at 121°C for 15 minutes. The broth was cooled for 24 hours at room temperature. A sterile inoculating loop was used to transfer MRSA from the agar slant to the broth. The MRSA broth was then incubated for 48 hours.

Inoculation of Artificial Turf

On day 1 of sampling, the MRSA broth was poured into an aluminum pan measuring 20.32x10.79x5.72-cm. Artificial turf from 2 of the plots was inoculated with MRSA broth. One swath of artificial turf was removed from each inoculation plot using sterile forceps. The swath was placed in the MRSA broth with the turf side downward. The swath was left in the broth for 5 seconds, lifted by the forceps and replaced into the space from which it was removed in the plot. Each swath was inoculated one at a time until all 16 swaths in each plot were inoculated. Remaining broth was autoclaved and disposed in biohazard waste. Inoculated plots were labeled outdoor environment inoculated (OEI) and laboratory inoculated (LI).

Exposure to Outdoor Environmental Conditions

One control plot and the outdoor environment inoculated plot were taken to a third floor exterior patio. The plots were transported with biohazard bags under each plot to prevent contamination during transportation. Additional biohazard bags were placed on the ground under the plots to collect run-off caused by precipitation during the trial. Once the plots had been placed on the patio, swath number 1 from the inoculated plot was removed from the plot using sterile forceps and placed in a plastic zip-lock bag labeled outdoor environment inoculated (OEI). Swath number 1 was removed from the control plot using sterile forceps and placed in separate plastic zip-lock bag labeled outdoor environment control (OEC). Sampling timeline is described in Figure 3. Timeline is repeated for all plots. Swaths were removed every 24 hours for a total of 7 days. Surface temperature, ambient temperature, precipitation, humidity, and solar radiation were measured at the time of sampling and recorded.

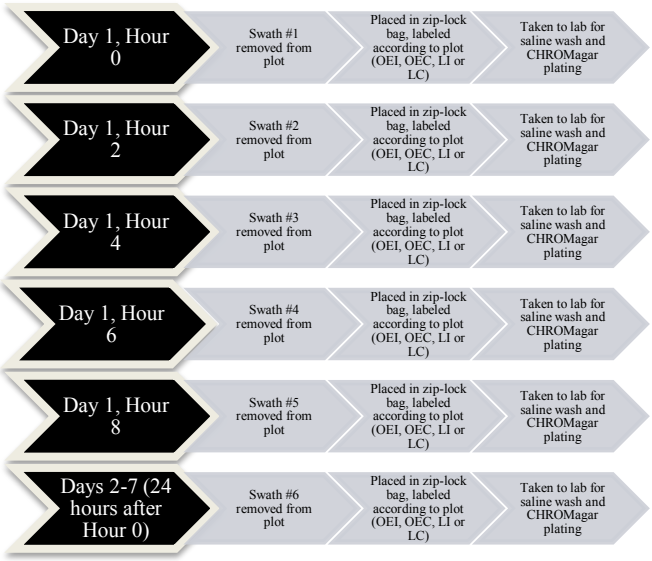

Figure 2. MRSA sampling timeline

Exposure to Laboratory Environment

One control plot and the lab inoculated plot remained in the lab. The plots were placed in the fume hood. Biohazard bags were placed under the plots to collect any potential runoff. Once the plots were placed in the fume hood, swath number 1 from each plot was removed using sterile forceps and placed in separate plastic zip-lock bags, labeled laboratory inoculated (LI) and laboratory control (LC) respectively. These samples were recorded at time 0. The sampling timeline was identical to that described for the environment plots in Figure 3. Surface temperature, ambient temperature, precipitation, and humidity were measured at the time of sampling and recorded.

Sampling of Swaths

A swath was removed from its transportation bag using sterile forceps and was placed in a 500mL beaker. The swath was washed by pouring 20mL of phosphate buffered saline over the swath. Run-off was collected in a beaker. A sterile cotton tipped applicator was dipped into the saline run-off. The applicator was swabbed over a quartered section of a CHROMagar plate. CHROMagar plates were divided in quarters and were labeled as outdoor environment inoculated (OEI), outdoor environment control (OEC), lab inoculated (LI) or lab control (LC) with the trial, and day of collection. All swaths were sampled using the same procedure as outlined above. CHROMagar plates were monitored at 24 and 48 hours. At 48 hours CHROMagar plates were recorded as positive or negative for MRSA presence and placed in a biohazard bag.

Data Analysis

Data collected were analyzed in SPSS 18.0 descriptively. Mean, median and standard deviation of survival times were determined with sample size n=4 for each trial. Mean, median, and standard deviation calculations were completed for surface temperature, ambient temperature, relative humidity, precipitation and solar radiation, grouped by sample plot (OEI, OEC, LI, LC) and trial.

CHAPTER 4: RESULTS

The purpose of this chapter is to describe the data collected across three sampling trials, specifically MRSA survival time on artificial turf in laboratory conditions as compared to outdoor environmental conditions. Environmental conditions were recorded for both outdoor and laboratory plots and are described here.

Survival of MRSA on Artificial Turf

To answer our research question, descriptive statistics were computed using SPSS 18.0. Mean, standard error, median, maximum and minimum values were calculated for MRSA survival, surface temperature, ambient temperature, relative humidity, precipitation and solar radiation.

The research question stated in Chapter 1 reads, "Are there differences in the viability of MRSA on artificial turf between laboratory conditions and outdoor environmental conditions?"

Our data indicated a mean difference between conditions. The mean survival of MRSA in laboratory conditions was 7.00 ± 0.00 days, with a median of 7 days. In an outdoor environment the mean survival was 4.67 ± 2.52 days, with a median of 5 days. Maximum and minimum values are presented in Table 1. Survival of MRSA on each plot is described in Table 2.

Table 1

Mean Survival of MRSA on Artificial Turf

	Mean ± SE	Maximum	Minimum
OEI	4.67±2.52	7.00	2.00
LI	7.00±0.00	7.00	7.00

OEI= Environmental Inoculated plot; LI= Laboratory Environment Inoculated Plot

Table 2

MRSA Survival on Artificial Turf by Plot

		Days
Trial 1	OEI	5.00
	OEC	0.00
	LI	7.00
	LC	0.00
Trial 2	OEI	7.00
	OEC	0.00
	LI	7.00
	LC	0.00
Trial 3	OEI	2.00
	OEC	0.00
	LI	7.00
	LC	0.00

OEI= Outdoor Environmental Inoculated Plot; OEC= Outdoor Environment Control Plot; LI= Laboratory Environment Inoculated Plot; LC= Laboratory Environment Control Plot

Environmental Conditions

Surface temperature, ambient temperature, relative humidity, precipitation and intensity of incoming solar radiation were measured and recorded daily. Precipitation did not occur in the laboratory setting. Intensity of incoming solar radiation was not measured for the laboratory setting; however, the fluorescence of the room lighting was 32 W. The control plots were placed in a fume hood out of direct fluorescent light.

Surface Temperature

Mean surface temperatures of all plots across all trials were 21.18 ± 4.92°C. Maximum surface temperature was 37°C with a minimum of 10°C. The mean surface temperature of outdoor environmental plots was 21.97 ± 6.48°C, with a maximum of 37°C and a minimum of 10°C. The mean surface temperature of laboratory plots was 20.38 ± 2.29°C, with a maximum of 26°C and a minimum of 14°C. Mean surface temperatures for individual plots and trials are described in Table 3.

Table 3

Mean Surface Temperature of Artificial Turf Plots

		Mean ± SD	Maximum	Minimum
Trial 1	OEI	21.00±5.88	27.00	10.00
	OEC	22.00±6.65	30.00	10.00
	LI	19.91±2.26	22.00	14.00
	LC	22.09±2.59	26.00	19.00
Trial 2	OEI	23.81±5.65	37.00	17.00
	OEC	23.73±6.10	35.00	12.00
	LI	18.09±2.02	21.00	15.00
	LC	19.73±0.79	21.00	19.00
Trial 3	OEI	20.09±7.12	31.00	12.00
	OEC	21.18±7.86	32.00	12.00
	LI	20.30±1.89	23.00	18.00
	LC	22.18±0.75	23.00	21.00

OEI= Outdoor Environmental Inoculated Plot; OEC= Outdoor Environment Control Plot; LI= Laboratory Environment Inoculated Plot; LC= Laboratory Environment Control Plot

Ambient Temperature

Mean ambient temperature across of all plots across trials was 18.05 ± 5.34°C. Maximum ambient temperature was 25.70°C with a minimum of 3.90°C. The mean ambient temperature of outdoor environmental plots was 14.54 ± 5.16°C, with a maximum of 25°C and a minimum of 3.90°C. The mean ambient temperature of laboratory plots was 21.55 ± 2.42°C, with a maximum of 25.70°C and a minimum of 14.60°C. Mean ambient temperatures for individual plots and trials are described in Table 4.

Table 4

Mean Ambient Temperature of Artificial Turf Plots

		Mean ± SD	Maximum	Minimum
Trial 1	OE	15.45±6.61	25.00	6.10
	LE	21.12±3.90	25.70	14.60
Trial 2	OE	11.49±3.58	15.00	3.90
	LE	20.74±1.00	22.60	19.40
Trial 3	OE	16.67±3.71	24.90	12.60
	LE	22.81±0.40	23.80	22.20

OE= Outdoor Environment Plots; LE= Laboratory Environment Plots

Relative Humidity

Mean relative humidity across of all plots across trials was 43.97 ± 17.15%. Maximum relative humidity was 91% with a minimum of 21%. The mean relative humidity of outdoor environmental plots was 51.24 ± 9.17%, with a maximum of 91% and a minimum of 24%. The mean relative humidity of laboratory plots was 36.70 ± 10.88%, with a maximum of 60% and a minimum of 21%. Mean relative humidity for individual plots and trials are described in Table 5.

Table 5

Mean Relative Humidity of Artificial Turf Plots

		Mean ± SD	Maximum	Minimum
Trial 1	OE	58.73±17.26	88.00	31.00
	LE	43.82±8.15	60.00	32.00
Trial 2	OE	37.00±12.03	68.00	24.00
	LE	25.00±4.890	37.00	21.00
Trial 3	OE	58.00±20.40	91.00	36.00
	LE	41.27±8.06	56.00	31.00

OE= Outdoor Environment Plots; LE= Laboratory Environment Plots

Precipitation

No precipitation occurred on laboratory samples; therefore, data represented are from outdoor environmental condition samples only. Mean precipitation across all trials was 0.58 ± 0.69cm. Maximum precipitation was 1.80cm with a minimum of 0.00cm. Mean precipitation for individual plots and trials is described in Table 6.

Table 6

Mean Precipitation of Artificial Turf Plots

		Mean ± SD	Maximum	Minimum
Trial 1	OE	0.86±0.69	1.80	0.00
Trial 2	OE	0.00±0.00	0.00	0.00
Trial 3	OE	0.97±0.64	1.40	0.00

OE= Outdoor Environment Plots

Solar Radiation

Solar radiation was not measured for laboratory samples; therefore, data is from outdoor environmental condition samples only. Mean solar radiation across all three trials was 310.42 ± 218.31WM^2. Maximum solar radiation was 710 WM^2 with a minimum of 0.00 WM^2. Mean solar radiation for individual plots and trials is described in Table 7.

Table 7

Mean Solar Radiation of Artificial Turf Plots

		Mean ± SD	Maximum	Minimum
Trial 1	OE	341.82±164.37	550.00	120.00
Trial 2	OE	468.55±224.43	710.00	0.00
Trial 3	OE	120.91±97.00	260.00	0.00

OE= Outdoor Environment Plots

Conclusion

We found a difference between the survival in days of MRSA in an outdoor environment and a laboratory environment. MRSA survived for an average of 4.67 ± 2.52 days in an outdoor environment and 7.00 ± 0.00 days in a laboratory environment. Environmental conditions were recorded but could not definitively be associated with viability. Possible effects of environmental conditions on viability of MRSA are discussed in Chapter 5.

CHAPTER 5: DISCUSSION

Our findings demonstrated a difference between the viability of MRSA on artificial turf in an outdoor environment and a laboratory environment. MRSA survived an average of 4.67 ± 2.52 days in an outdoor environment and 7.00 ± 0.00 days in a laboratory environment.

Survival of MRSA in a Laboratory Setting

Previous studies have concluded that MRSA can survive on artificial turf in a laboratory setting for an unlimited amount of time when provided a nutrient base, such as mucin.[36] Our research provided MRSA with a residual nutrient supply, carried over during the inoculation process. Our data showed MRSA survived for 7 days at which time the trial was ended.

MRSA may have the capability of surviving for a longer period of time in conditions, such as those in a laboratory, where temperature, relative humidity, and solar radiation do not fluctuate a great deal or peak at extreme values. Little evidence exists specifying the necessary surface temperature, ambient temperature, relative humidity, or solar radiation to support MRSA survival in a laboratory environment.

There was little variation in survival across the trials, even with shifts in environmental conditions. Laboratory conditions did not place enough stress on the bacteria to cause total MRSA death. Although MRSA in laboratory conditions survived the maximum number of observed days, conditions were not constantly ideal. Optimal conditions for MRSA survival are ambient environmental and surface temperatures at 25°C and 11-33% relative humidity.[5]

Surface temperatures on laboratory plots ranged from 26.00°C (trial 1) to 14.00°C (trial 1), with a mean of 20.38 ± 2.29°C. The mean and low end ranges are below optimal temperatures for MRSA survival.[5] Additional environmental conditions within optimal range[5] may have allowed for continued survival of the bacteria. Surface temperatures were not increased by solar radiation as the plots were located in a fume hood for the duration of the trial.

Laboratory ambient temperatures ranged from 25.70°C (trial 1) to 14.60°C (trial 2), with a mean of 21.55 ± 2.42°C, occasionally reaching optimal temperature for MRSA survival.[5] Ambient environmental and surface temperatures remained within ±5.30°C during sampling. Ambient temperature was controlled by a thermometer within the laboratory and was not manually adjusted during the sampling period.

Laboratory relative humidity ranged from 60% (trial 1) to 21% (trial 2), with a mean of 36.70 ± 10.88%. Optimal relative humidity for MRSA survival is 11-33%.[5] Relative humidity fell within the optimal range[5] during trial 1 (day 5), trial 2 (days 1, 2, 5, 6, and 7) and trial 3 (day 1, time 0 and 2 and day 5). Relative humidity above optimal conditions[5] was not effective in limiting MRSA survival during any of the 7-day trials. Additional environmental factors are required to negatively affect MRSA survival.

Precipitation was not recorded for laboratory samples. No external fluid accumulated on the artificial turf during the trial periods.

Solar radiation was not monitored in the lab due to placement of the sample plots in the fume hood. This location decreased the direct ultraviolet (UV) light exposure on the plots. The wattage of the light bulbs in use in the lab is 32 W. Solar radiation,

including UV light, appears to have minimal effect of MRSA survival in a laboratory setting in previous studies.[36] Solar radiation from lab lighting had minimal effect on surface temperature changes and no apparent effect on MRSA survival during the trials.

Survival of MRSA in an Outdoor Environmental Setting

There are no data available detailing the viability of MRSA on artificial turf in outdoor environmental conditions. Conditions in an outdoor environment are constantly changing, unlike a lab where the climate is more likely to be stable. A variety of environmental conditions provide opportunities for homeostatic conditions for MRSA survival. Extreme conditions may decrease MRSA viability.

MRSA is capable of surviving a variety of conditions according to our data. During the trial the MRSA survived frost conditions, heavy precipitation, heat, high relative humidity, and solar radiation. It cannot be assumed that MRSA does not survive simply because the climate is outside of the normal homeostatic conditions, because multiple environmental factors affect survival.

Surface temperatures on outdoor environment plots ranged from 37.00°C (trial 2) to 10.00°C (trial 1), with a mean of 21.97 ± 6.48°C. Surface temperatures were consistently higher than ambient temperatures in the outdoor setting, suggesting that solar radiation assisted the artificial turf surface in reaching higher temperatures and allowing surface temperatures to remain closer to the optimal survival temperature during the trials. The outdoor environment may have provided temperatures closer to those preferred for MRSA survival, assisted by environmental factors, specifically solar radiation.

Ambient temperatures of outdoor environmental plots ranged from 25.00°C (trial 1) to 3.90°C (trial 2), with a mean of 14.54 ± 5.16°C. Ambient temperatures were consistently lower than surface temperatures. On trial 2, day 2 of sampling, the ambient temperature was 25.90°C lower than the surface temperature measurement. Lower environmental temperatures may allow for additional heating by solar radiation, increasing the surface temperature to optimal conditions[5] and supporting MRSA survival. Cold ambient temperature readings, such as trial 2, day 6 (3.90°C) may permit MRSA survival due to the effect solar radiation may have on the surface temperature.

Relative humidity of outdoor environmental plots ranged from 91% (trial 3) to 24% (trial 2), with a mean of 51.24 ± 9.17%. Trial 2 had the lowest mean relative humidity (37.00 ± 12.03%) and the highest survival rate (7 days) although the mean is above the optimal percentage.[5] Previous studies have shown that higher relative humidity leads to decreased MRSA survival.[5] Data collected during this research supports this claim. Continued research is required to determine the individual effect of this environmental condition on MRSA survival.

Precipitation on outdoor environmental plots ranged from 1.80cm (trial 1) to 0.00cm (all trials), with a mean of 0.58 ± 0.69cm. Although the maximum amount of precipitation occurred during trial 1, precipitation may have primarily affected trial 3. Precipitation occurred during day 1, between the hour 2 and hour 4 sample times. This was the earliest sample time affected by precipitation in a trial. Residual nutrients deposited with the MRSA bacteria during inoculation may have been washed off at this time, depleting the nutrient source necessary for survival on day 1 of the trial. This

example supports existing research stating that MRSA requires a nutrient source in order to survive on artificial turf.[36]

Solar radiation on the outdoor environmental plots ranged from 710 WM^2 (trial 2) to 0.00 WM^2 (trials 2 and 3), with a mean of 310.42 ± 218.31WM^2. This study was not focused on providing MRSA protection from solar radiation, including ultraviolet radiation; therefore, formation of biofilms prior to MRSA application on to artificial turf was not monitored or supported. Increased ultraviolet light decreases the survival of MRSA[5, 23]; however, in this study the trial with the highest mean (468.55 ± 224.43WM^2) survived the longest period of time and the lowest mean (120.91 ± 97.00WM^2) survived the shortest period of time. Solar radiation is not interchangeable with UV light is this study. The amount of UV light emitted during data collection was not determined. Solar radiation data collected during the trials cannot be considered contrary to current research concerning viability of MRSA and UV light exposure. Solar radiation may have supported the survival of MRSA by increasing the surface temperature of the artificial turf to temperatures acceptable for survival.

Effect of Multiple Outdoor Environmental Conditions on MRSA Survival

Multiple variables affect the survival of MRSA in an outdoor environment. We were unable to determine a combination of conditions causing an increase or decrease in survival; however, data from trial 2 provides an example of how survival could be affected by environmental conditions as a whole versus a specific conditions or a specific combination of conditions. Trial 2 experienced optimal surface temperatures,[5] below optimal ambient temperatures[5], relative humidity in an optimal range,[5] 200-700WM^2

solar radiation and no precipitation. Solar radiation may have increased the surface temperatures, creating more probable conditions for MRSA survival. Lack of precipitation may have allowed MRSA to use all available nutrients as a food source. Maximum MRSA survival in an outdoor environment occurred during this trial. A specific set of conditions required to alter MRSA survival could not be determined in this research.

Limitations

This research was collected in Athens, OH in March and April, 2012. Environmental conditions occurring during the study are limited to those naturally occurring at the site. Exact environmental conditions may be difficult to replicate. Environmental conditions were measured at the time of sample collection. The conditions were not continually recorded, but were measured every 24 hours during the trial periods.

Clinical Application

The MRSA bacteria are capable of survival on artificial turf in an outdoor environment in a variety of environmental conditions. Artificial turf surfaces are used across the globe in a variety of climates, with varying ability to support or reduce the survival of MRSA. In conditions similar to those experienced during the trial, MRSA is capable of surviving 4.67 ± 2.52 days on artificial turf in an outdoor environment.

Laboratory settings are capable of supporting MRSA indefinitely when provided with nutrients.[36] Artificial turf supports MRSA survival for 7 days without supplemented nutrients. Indoor multipurpose artificial turf facilities may have conditions similar to those found in laboratory conditions, allowing MRSA survival for 7 days without added

nutrients. The risk of infection in indoor multipurpose facilities may be overlooked, suggesting the need to test MRSA survival on artificial turf in an indoor facility.

If bacteria are deposited on the artificial turf at a high school during a practice on Thursday and the high school supports football, boys' and girls' soccer, and field hockey, multiple MRSA exposures may occur over the next 7 days, including exposures during three football games. Football has the highest incidence of turf abrasions and facilitation of infection.[2, 13, 18, 47] Additional events involving contact with the artificial turf may increase the exposure risk. Proper field and wound management will decrease the risk of infection during participation on artificial turf.

The National Athletic Trainers' Association position statement[4] on skin diseases states that athletes who present with MRSA infections are able to continue participation in sport as long as the affected area is covered. Bandages may not cover the entire lesion or may fall off during participation, potentially exposing fellow athletes to skin to skin contact with the lesion and increasing infection risk. Drainage from the lesion could also infect the artificial turf. Proper enforcement of secure wound coverage above and below the lesion as well as rational restriction from play if the wound cannot be properly covered will decrease skin to skin transfer and deposits on artificial turf.

MRSA can also be deposited on artificial turf via nasal secretions or saliva, exposing MRSA to the environment as well as providing a nutrient source for MRSA survival.[36] MRSA has been found in the nostrils of athletes, with up to 30% of the population carrying the bacteria, and can be deposited on artificial turf via nasal secretions.[1, 18, 23] Enforcement of a no spitting or open nose blowing rule may decrease

MRSA infections my eliminating a vital nutrient source for the bacteria, decreasing viability.

MRSA has been observed to infect athletic teams[3, 6, 7, 11, 13-30] and will continue to be present on artificial turf, if clear precautions are not followed. Knowledge of MRSA transmission and viability in indoor and outdoor environments allows athletic trainers to do their best to limit the number of exposures and be mindful of prime MRSA survival periods.

Future Research

Future research should examine the individual effect of each environmental condition of the survival of MRSA in an outdoor environment. Additional research conducted during temperatures occurring during high use periods such as late summer and fall would be useful in attempting to decrease MRSA infections.

REFERENCES

1. Gorwitz RJ, Jernigan DB, Powers JH, et al; Centers for Disease Control and Prevention-Convened Experts' Meeting on Management of MRSA in the Community. *Strategies for Clinical Management of MRSA in the Community* [Summary]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Prevention and Control; 2006. http://www.cdc.gov/mrsa/pdf/MRSA-Strategies-ExpMtgSummary-2006.pdf. Accessed May 2, 2012.

2. Cohen PR. Cutaneous community-acquired methicillin-resistant *Staphylococcus aureus* infection in participants of athletic activities. *South Med J*. 2005;98:596-602.

3. Kurkowski C. CA-MRSA: The new sports pathogen. *Orthop Nurs*. 2007;26:310-314.

4. Zinder SM, Baster RS, Foley J, Scarlata C, Vasily DB. National Athletic Trainers Association position statement: skin diseases. *J Athl Train*. 2010;45:411-428.

5. Cimolai N. MRSA and the environment: implications for comprehensive control measures. *Eur J Clin Microbiol Infect Dis*. 2008;27:481-493.

6. Cohen PR. Community-acquired methicillin-resistant *Staphylococcus aureus* skin infections: implications for patients and practitioners. *Am J Clin Dermatol*. 2007;8:259-270.

7. Levenhagen KM. Community-acquired methicillin-resistant *Staphylococcus aureus*: An emerging concern for physical therapists: Discussion. *Physiother Res Intl.* 2008;13:9-17.

8. Weber JT. Community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis*. 2005;41:S269-S272.

9. Grindstaff TL, Saliba SA, Mistry DJ, MacKnight JM. Community-associated methicillin-resistant *Staphylococcus aureus*. *N Am Sport Phys Ther*. 2007;2:139-146.

10. Davis SL, Perri MB, Donabedian SM, et al. Epidemiology and outcomes of community-associated methicillin-resistant *Staphylococcus aureus* infection. *J Clin Microbio.* 2007;45:1705-1711.

11. Stanforth B, Krause A, Starkey C, Ryan TJ. Prevalence of community-associated methicillin-resistant *Staphylococcus aureus* in high school wrestling environments. *J Environ Health*. 2010;72:12-16.

12. Anderson BJ. Skin infections in combat sports. In: Kordi, R, Maffuli N, Wroble RR, Wallace WA, eds. *Combat Sports Medicine*. 1st ed. London, UK: Springer-Verlag; 2009:121-133.

13. American Academy of Dermatology. Athletes prone to a rash of skin conditions. *Sci Daily*. 2011. http://www.sciencedaily.com /releases/2011/02/110205140049.htm. Accessed April 1, 2012.

14. Archibald LK, Shapiro J, Pass A, Rand K, Southwick F. Methicillin-resistant *Staphylococcus aureus* infection in a college football team: risk factors outside the locker room and playing field. *Infec Control Hosp Epidemiol*. 2008;29:450-453.

15. Barrett TW, Moran GJ. Update on emerging infections: news from the Centers of Disease Control and Prevention. *Ann Emerg Med*. 2004;43:43-47.

16. Beam J, Buckley B. Community-acquired methicillin-resistant *Staphylococcus aureus*: prevalence and risk factors. *J Athl Train*. 2006;41:337-340.

17. Begier E, Frenette K, Barrett N, et al. A high morbidity outbreak of methicillin-resistant *Staphylococcus aureus* among players on a college football team,

facilitated by cosmetic body shaving and turf burns. *Clin Infect Dis*. 2004;39:1446-1453.

18. Buss BF, Mueller SW, Theis M, Keyser A, Safranek TJ. Population-based estimates of methicillin-resistant *Staphylococcus aureus* (MRSA) infections among high school athletes–Nebraska, 2006-2008. *J Sch Nurs*. 2009;25:282-291.

19. Kazakova S, Hageman J, Matava M, et al. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. *N Engl J Med*. 2005;35:468-475.

20. Kirkland EB, Adams BB. Methicillin-resistant *Staphylococcus aureus* and athletes. *J Am Acad Dermatol*. 2008;59:494-502.

21. Lear A, McCord G, Peiffer J, Watkins RR, Parikh A, Warrington S. Incidence of *Staphylococcus aureus* nasal colonization and soft tissue infection among high school football players. *J Am Board Fam Med*. 2001;24:429-435.

22. Lindenmayer JM, Schoenfeld S, O'Grady R, Carney JK. Methicillin-resistant *Staphylococcus aureus* in a high school wrestling team and the surrounding community. *Arch Intern Med*. 1998;158:895-899.

23. Many LS. Preventing community-associated methicillin-resistant *Staphylococcus aureus* among student athletes. *J Sch Nurs*. 2008;24:370-378.

24. Montgomery K, Ryan TJ, Krause A, Starkey C. Assessment of athletic health care facility surfaces for MRSA in the secondary school setting. *J Environ Health*. 2010;72:8-11.

25. Nguyen DM, Mascola L, Bancroft E. Recurring methicillin-resistant *Staphylococcus aureus* infections in a football team. *Emerg Infect Dis*. 2005;11:526-532.

26. Oller AR, Province L, Curless B. *Staphylococcus aureus* recovery from environmental and human locations in 2 collegiate athletic teams. *J Athl Train*. 2010;45:222-229.

27. Redziniak DE, Diduch DR, Turman K, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in the athlete. *Int J Sports Med*. 2009;30:557-562.

28. Rihn JA, Michaels MG, Harner CD. Community-acquired methicillian-resistant *Staphylococcus aureus*: an emerging problem in the athletic population. *Am J Sports Med*. 2005;33:1924-1929.

29. Romano R, Lu D, Holtom P. Outbreak of community-acquired methicillin-resistant *Staphylococcus aureus* skin infections among a collegiate football team. *J Athl Train*. 2006;41:141-145.

30. Sanders JC. Reducing MRSA infections in college student athletes: implementation of a prevention program. *J Com Health Nurs*. 2009;26:161-172.

31. Cohen PR. The skin in the gym: a comprehensive review of the cutaneous manifestations of community-acquired methicillin-resistant *Staphylococcus aureus* infection in athletes. *J Clin Dermatol*. 2008;26:16-26.

32. Stevens MP, Bearman G, Rosato A, Edmond M. Community-acquired methicillin-resistant *Staphylococcus aureus* in a women's collegiate basketball team. *South Med J*. 2008;101:1067-1068.

33. Adams BB. Dermatologic disorders of the athlete. *Sports Med*. 2002;32:309-321.

34. Coughenour C, Stevens V, Stetcenbach LD. An evaluation of methicillin-resistant *Staphylococcus aureus* survival on five environmental surfaces. *Microbiol Drug Resistance.* 2011;17:457-461.

35. Garza D, Sungar G, Johnston T, Rolston B, Ferguson JD, Matheson GO. Ineffectiveness of surveillance to control community-associated methicillin-

resistant *Staphylococcus aureus* in a professional football team. *Clin J Sport Med*. 2009;19:498-501.

36. Waninger KN, Rooney TP, Miller JE, Berberian J, Fujimoto A, Buttaro BA. Community-associated methicillin-resistant *Staphylococcus aureus* survival on artificial turf substrates. *Med Sci Sports Exerc*. 2010;43:779-784.

37. National Athletic Trainers' Association (NATA). Official Statement from the National Athletic Trainers' *Association on communicable and infectious diseases in secondary school sports*; 2007. http://www.nata.org/sites/default/files/CommunicableInfectiousDiseasesSecondarySchoolSports.pdf. Accessed March 1, 2012.

38. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *J Am Med Assoc*. 2007;298:1763-1771.

39. Brown DJ, Edwards DI, Hawkey PM, et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *J Antimicrob Chemother*. 2005;56:1000-1018.

40. Zinder SM, Baster RS, Foley J, Scarlotta C, Vasily DB. National Athletic Trainers Association position statement: skin diseases. *J Athl Train*. 2010;45:411-428.

41. Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. *Clin Infect Dis*. 2003;36:1433-1437.

42. Matouskova I, Janout V. Current knowledge of methicillin-resistant S*taphylococcus aureus* and community-associated methicillin-resistant *staphylococcus aureus*. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2008;150:191-202.

43. Moore CL, Hingwe A, Donabedian SM, et al. Comparative evaluation of epidemiology and outcomes of methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 infections causing community- and healthcare-associated infections. *International J of Antimicrobial Agents*. 2009;34:148-155.

44. Milstone A, Carroll K, Ross T, et al. Community-associated methicillin-resistant *Staphylococcus aureus* strains in pediatric intensive care unit. *Emerg Infect Dis*. 2010;16:647-655.

45. Del Giudice P, Blanc V, Durupt F, et al. 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 J of Derm*. 2006;154:118-124.

46. Shenoy M, Bhat G, Kishore A, Hassan MK. Significance of MRSA strains in community associated skin and soft tissue infections. *Indian J Med Microbiol*. 2010;28:152-154.

47. Benjamin HS, Nikore V, Takagishi J. Practical management: community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA): the latest sports epidemic. *Clin J Sports Med*. 2007;17:393-397.

48. Purcell K, Fergie J. Epidemic of community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Arch Pediatr Adolesc Med*. 2005;159:980-985.

49. Spoering AL, Lewis K. Biofilms and planktonic cells of psuedomunas aeruginosa have similar resistance to killing by antimicrobials. *J Bacteriol*. 2001;183:6746-6751.

50. Michels HT, Noyce JD, Keevil CW. Effects of temperature and humidity on the efficacy of methicillin-resistant *Staphylococcus aureus* challenged antimicrobial materials containing silver and copper. *Society Applied Microbio.* 2009;49:191-195.

51. Flayhart D, Hindler J, Bruckner D, et al. Multicenter evaluation of BBL CHROMager MRSA medium for direct detection of methicillin-resistant *Staphylococcus aureus* for surveillance culters of the anterior nares. *J Clin Microbiol*. 2005;5536-5540.

52. Pape J, Wadlin J, Nachamkin I. Use if BBL CHROMagar MRSA medium for identification of methicillin-resistant *Staphylococcus aureus* directly from blood cultures. *J Clin Microbiol.* 2006;44:2575-2576.

53. Becton, Dickenson & Company. Product center: BBL™ CHROMagar ™ MRSA. 2008. http://www.bd.com/ds/productCenter/215084.asp. Accessed March 1, 2012.

54. Elston DM. Community-acquired methicillin-resistant *Staphylococcus aureus*. *J Am Acad Dermatol.* 2007;56:1-16.

55. Stevens MP, Bearman G, Rosato A, Edmond M. Community-acquired methicillin-resistant *Staphylococcus aureus* in a women's collegiate basketball team. *South Med J*. 2008;101:1067-1068.

56. Turbeville SD, Cowan LD, Greenfield RA. Infectious disease outbreaks in competitive sports. *American J of Sports Med.* 2006;34:1860-1865.

57. Kluytumans JA, Wertheim HF. Nasal carriage of *Staphylococcus aureus* and prevention of nosocomial infections. *Infection*. 2005;33:3-8.

58. Stiles VH, James IT, Dixon SJ, Guisasola IN. Natural turf surfaces. *Sports Med*. 2009;39:65-84.

59. Roberts MC, Soge O, No D, Helgeson SE, Meschke JS. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from public surfaces on a university campus, student homes and local community. *J Appl Microbiol*. 2011:110:1531-1537.

APPENDIX A: IBC APPROVAL

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May 14, 2012

To: Timothy J. Ryan, Professor, Social and Public Health
From: IBC
Subject: PROPOSAL REVIEW

Your IBC protocol has been approved by the IBC, with the approval number M B-2-1101. This is a tracking number used by the IBC.

MB-2-1101 is approved for five years, until 2016.

Please note the items checked off below.
If you have any questions, please call David Schleter at 593-1662. Thank You!

Remember, this approval only applies to the information submitted on your form. Changes in the agents or Biosafety Level's (BSL) will require an addendum or new form to be submitted and approval obtained in the future.

The following are also required:

X 1. A Bloodborne Pathogens Program is required. – Already in place

X 2 Proposals ≥ BSL2 require a signature. A copy of your protocol will be sent to you in campus mail. Please sign the form in the indicated spot and return it by campus mail to: Biosafety Officer/EHS/Hudson Health Center.

☐ 3. Other (Specify:)

APPENDIX B: RAW DATA

Trial	Plot	Sample	Surface Temperature (°C)	Ambient Temperature (°C)	Relative Humidity (%)	Solar Radiation (WM²)	Precipitation (cm)	CHROMagar at 24h	CHROMagar at 48h
1	1	1	20	18.90	51	550	0	1	1
1	1	2	24	21.10	51	190	0	1	1
1	1	3	21	20.00	74	250	0	1	1
1	1	4	24	25.00	43	450	0	1	1
1	1	5	25	23.90	31	200	0.508	1	1
1	1	6	27	11.10	57	540	0.508	1	1
1	1	7	10	6.10	88	430	0.7112	1	1
1	1	8	11	8.80	80	120	1.6002	2	2
1	1	9	18	10.00	69	200	1.6002	1	1
1	1	10	24	10.00	57	550	1.8034	2	2
1	1	11	27	15.00	45	280	1.8034	2	2
1	2	1	25	18.90	51	550	0	1	1
1	2	2	30	21.10	51	190	0	2	2
1	2	3	21	20.00	74	250	0	2	2
1	2	4	28	25.00	43	450	0	2	2
1	2	5	26	23.90	31	200	0.508	2	2
1	2	6	25	11.10	57	540	0.508	2	2
1	2	7	10	6.10	88	430	0.7112	2	2
1	2	8	11	8.80	80	120	1.6002	2	2
1	2	9	18	10.00	69	200	1.6002	2	2
1	2	10	21	10.00	57	550	1.8034	2	2
1	2	11	27	15.00	45	280	1.8034	2	2
1	3	1	20	23.00	44	0	0	1	1
1	3	2	20	23.30	47	0	0	1	1
1	3	3	22	24.80	51	0	0	1	1
1	3	4	21	24.90	48	0	0	1	1
1	3	5	22	25.70	41	0	0	1	1
1	3	6	22	22.80	32	0	0	1	1
1	3	7	14	19.00	40	0	0	1	1
1	3	8	19	14.60	48	0	0	1	1
1	3	9	20	14.70	60	0	0	1	1
1	3	10	19	19.30	37	0	0	1	1
1	3	11	20	20.20	34	0	0	1	1
1	4	1	23	23.00	44	0	0	2	2
1	4	2	23	23.30	47	0	0	2	2
1	4	3	25	24.80	51	0	0	1	1

1	4	4	25	24.90	48	0	0	2	2
1	4	5	26	25.70	41	0	0	2	1
1	4	6	23	22.80	32	0	0	2	2
1	4	7	19	19.00	40	0	0	2	2
1	4	8	19	14.60	48	0	0	2	2
1	4	9	20	14.70	60	0	0	2	2
1	4	10	20	19.30	37	0	0	2	2
1	4	11	20	20.20	34	0	0	2	2

Trial	Plot	Sample	Surface Temperature (°C)	Ambient Temperature (°C)	Relative Humidity (%)	Solar Radiation (WM²)	Precipitation (cm)	CHROMagar at 24h	CHROMagar at 48h
2	1	1	24	7.70	42	520	0	1	1
2	1	5	20	15.00	24	220	0	1	1
2	1	6	37	11.10	35	540	0	1	1
2	1	7	24	15.00	33	560	0	1	1
2	1	8	27	12.70	42	710	0	2	2
2	1	9	13	7.80	38	510	0	2	2
2	1	10	23	3.90	68	0	0	1	1
2	1	11	23	14.50	40	234	0	1	1
2	2	1	28	7.70	42	520	0	2	2
2	2	2	20	12.20	31	700	0	2	2
2	2	3	20	12.70	28	660	0	2	2
2	2	4	24	13.80	26	500	0	2	2
2	2	8	31	12.70	42	710	0	2	2
2	2	9	12	7.80	38	510	0	2	2
2	2	10	24	3.90	68	0	0	2	2
2	2	11	23	14.50	40	234	0	2	2
2	3	1	18	21.00	23	0	0	1	1
2	3	2	15	19.70	23	0	0	1	1
2	3	3	16	19.40	23	0	0	1	1
2	3	4	16	20.40	22	0	0	1	1
2	3	5	16	20.30	21	0	0	1	1
2	3	6	19	20.70	23	0	0	1	1
2	3	7	20	20.50	37	0	0	1	1
2	3	11	21	22.50	24	0	0	1	1
2	4	1	20	21.00	23	0	0	2	2
2	4	2	19	19.70	23	0	0	2	2
2	4	3	19	19.40	23	0	0	2	2

2	4	4	19	20.40	22	0	0	2	2
2	4	5	20	20.30	21	0	0	2	2
2	4	6	19	20.70	23	0	0	2	2
2	4	7	20	20.50	37	0	0	2	2
2	4	8	19	20.60	32	0	0	2	2
2	4	9	20	20.40	23	0	0	2	2
2	4	10	21	22.60	24	0	0	2	2

Trial	Plot	Sample	Surface Temperature (°C)	Ambient Temperature (°C)	Relative Humidity (%)	Solar Radiation (WM²)	Precipitation (cm)	CHROMagar at 24h	CHROMagar at 48h
3	1	1	15	15.40	45	166	0	1	1
3	1	5	12	12.80	91	15	1.1938	1	1
3	1	6	20	19.40	57	153	1.397	1	1
3	1	7	31	24.90	45	237	1.397	2	2
3	1	8	23	16.10	38	260	1.397	2	2
3	1	9	28	16.50	36	244	1.397	2	2
3	1	10	24	12.80	81	104	1.397	2	2
3	1	11	28	20.40	52	0	1.397	2	2
3	2	1	17	15.40	45	166	0	2	2
3	2	2	18	16.10	44	52	0	2	2
3	2	3	12	16.40	58	81	0.0254	2	2
3	2	4	12	12.60	91	18	0.9906	2	2
3	2	8	26	16.10	38	260	1.397	2	2
3	2	9	30	16.50	36	244	1.397	2	2
3	2	10	22	12.80	81	104	1.397	2	2
3	2	11	32	20.40	52	0	1.397	2	2
3	3	2	18	22.9	32	0	0	1	1
3	3	3	19	22.2	42	0	0	1	1
3	3	4	19	22.8	44	0	0	1	1
3	3	5	18	22.8	44	0	0	1	1
3	3	6	22	22.7	45	0	0	1	1
3	3	7	23	22.9	56	0	0	1	1
3	3	8	22	23	35	0	0	1	1
3	4	1	23	22.7	31	0	0	2	2
3	4	2	22	22.9	32	0	0	2	2
3	4	3	22	22.2	42	0	0	2	2
3	4	4	23	22.8	44	0	0	2	2
3	4	5	21	22.8	44	0	0	2	2

3	4	6	22	22.7	45	0	0	2	2
3	4	7	23	22.9	56	0	0	2	2
3	4	8	22	23	35	0	0	2	2
3	4	9	22	22.7	31	0	0	2	2
3	4	10	21	22.4	46	0	0	2	2
3	4	11	23	23.80	48	0	0	2	2

Mean Environmental Conditions

		Surface Temperature (°C)	Environmental Temperature (°C)	Relative Humidity (%)	Solar Radiation (WM²)	Precipitation (cm)
Trial 1	OE	21.50±6.15	15.45±6.61	58.73±17.26	341.82±164.37	0.86±0.69
	LE	21.00±2.62	21.12±3.90	43.82±8.15	n/a	n/a
Trial 2	OE	23.77±5.74	11.49±3.58	37.00±12.03	468.55±224.43	0.00±0.00
	LE	18.91±1.72	20.74±1.00	25.00±4.89	n/a	n/a
Trial 3	OE	20.64±7.34	16.67±3.71	58.00±20.40	120.91±97.00	0.97±0.64
	LE	21.29±1.68	22.81±.40	41.27±8.06	n/a	n/a

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