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Community-Associated Methicillin-Resistant *Staphylococcus aureus* Survival on Artificial Turf Substrates

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[1]*Department of Family Medicine and Emergency Medicine, Temple University School of Medicine, Philadelphia, PA;* [2]*Sports Medicine Fellowship, St. Luke's Hospital and Health Network, Bethlehem, PA;* [3]*Department of Kinesiology, Temple University, Philadelphia, PA;* [4]*Delaware Valley College, Doylestown, PA; and* [5]*Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA*

ABSTRACT

WANINGER, K. N., T. P. ROONEY, J. E. MILLER, J. BERBERIAN, A. FUJIMOTO, and B. A. BUTTARO. Community-Associated Methicillin-Resistant *Staphylococcus aureus* Survival on Artificial Turf Substrates. *Med. Sci. Sports Exerc.*, Vol. 43, No. 5, pp. 779–784, 2011. **Objective**: Artificial turf has been suggested as a risk factor for community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). This is an experimental study looking at survival of CA-MRSA on artificial turf. **Methods**: MRSA strain USA-300-0114 was grown as either planktonic cells or biofilms in liquid cultures of beef heart infusion broth overnight at 37°C. Beakers containing ProGrass (Pittsburgh, PA) turf were inoculated at the dirt interface with either $\sim 5 \times 10^7$ planktonic bacteria or with biofilms. The inoculum included varying nutrient conditions consisting of spent medium, saline, or 5% mucin. The beakers were incubated at 37°C in ambient air. The main outcome measure was the number of surviving colony-forming units determined by plating on mannitol salt agar. **Results**: Survival was biphasic with a colony-forming unit drop from $\sim 5 \times 10^7$ to $\sim 5 \times 10^5$ after the first week followed by survival of between 10^4 and 10^3 bacteria until termination of the experiment (20–50 d). Survival was dependent on nutrients, and washed cells survived less than 1 d. Mucin could serve as a nutrient source and slightly increased surviving numbers to 10^4–10^5 bacteria. Biofilm formation did not influence survival. **Conclusions**: CA-MRSA survivability on artificial turf surfaces is dependent on the availability of nutrients. These results suggest that CA-MRSA could survive on artificial turf in significant numbers for 1 wk, and lower numbers for at least 1 month, if supplied with appropriate nutrients. Outdoor environmental conditions may affect these findings. **Key Words:** COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*, SPORTS, FOOTBALL, INFECTIOUS DISEASE, TURF FIELDS

Infections with community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) continue to be an emerging problem in the outpatient setting (13,21). Once associated mainly with immune-compromised patients, this organism has transitioned into the community and now threatens the young and the healthy. Those involved in athletic activities may be at increased risk of infection (3,20,32). Case series of outbreaks of CA-MRSA infections within sports teams have been reported (1,2,4–8,18,19,23,28,33, 34,36,37,40). These infections have become a source of significant morbidity and, on rare occasions, even mortality. Transmission occurs predominately through person-to-person spread, but indirect contact with contaminated items or surfaces has been reported (2,4,6,19). Common factors associated with outbreak transmission include crowding, frequent skin-to-skin contact, compromised skin integrity, shared items and contaminated surfaces, and lack of cleanliness. Individuals concerned with the management of artificial turf surfaces have called for additional research into the incidence, survivability, and transmission of CA-MRSA to athletes on artificial and natural turf surfaces (17). Those who compete in contact sports played on artificial turf surfaces have been identified

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as an at-risk group, as turf is generally thought to put athletes at increased risk of infection because of compromised skin integrity from turf abrasions (2,4–7,19,28,33,34).

The infectious risk associated with exposure to artificial turf has not been well studied, and it is not clear whether exposure to artificial turf may also put athletes at increased risk for direct infection from a contaminated turf surface. This is a prospective study evaluating the viability of CA-MRSA on artificial turf as well as time course of organism survival on these surfaces.

METHODS

Bacterial strains and culture conditions. CA-MRSA strains used in these studies were #167-05 MRSA, USA-300-0114 PVL+ SCCmecIV provided by the Centers for Disease Control (CDC). Strains were grown in beef heart infusion (BHI) broth or on mannitol salt agar. All cultures were incubated overnight at 37°C. All laboratory protocols were under the direct supervision of a doctorate-level microbiologist following predetermined protocols. The distribution of CA-MRSA on each sample was done in a uniform manner to maintain protocol consistency.

Planktonic cells. Strains were grown in broth cultures of BHI overnight at 37°C. For samples containing carryover nutrients, a 400-μL aliquot of overnight culture ($\sim 5 \times 10^7$ MRSA) was directly inoculated into the center of the beaker at the "dirt" surface. For samples without nutrients, the bacteria were harvested by centrifugation at 8000 rpm and washed three times with 0.9% NaCl. The cells were then resuspended in a volume of 0.9% NaCl equivalent to the starting volume. A sample containing $\sim 5 \times 10^7$ MRSA (400 μL) was directly inoculated into the center of the beaker at the "dirt" surface.

Biofilms. To determine whether biofilm formation affected survival of CA-MRSA under conditions of nutrient starvation, MRSA biofilms were grown on glass coverslips in 24-well microtiter plates in BHI. The coverslip was removed, and proper formation of the biofilm was confirmed by staining with fluorescent DNA stain SYTO 9 and imaged by confocal microscopy. The spent medium from the overnight culture was removed, and the biofilm was suspended in 400 μL of BHI (nutrients). For cultures with no nutrients, the biofilm was carefully washed and then resuspended in 400 μL of 0.9% NaCl (no nutrients). The biofilm cells from one coverslip were inoculated into the center of the turf beaker at the "dirt" surface. Because of the difficulty in disrupting biofilm cells, survival was scored as positive or negative, and colony-forming units (CFU) were not calculated.

Survival assays. Beakers (250 mL) containing ProGrass (Pittsburgh, PA) turf samples were prepared. The turf was not autoclaved to prevent destruction of the turf and possible release of compounds toxic to bacteria. Uninoculated artificial turf was tested as controls and did not grow any MRSA when plated on mannitol salt agar. By plating on mannitol salt agar, *S. aureus* cultures were easily isolated from any environmental contaminants. Each beaker was inoculated in the center at the "dirt" surface with 400-μL samples containing $\sim 5 \times 10^7$ CFU of planktonic MRSA or biofilms with or without nutrient carryover from the cultures. The beakers were then incubated at 37°C. At designated biweekly time points, a beaker was removed. For recovery of surviving planktonic cells, sterile 0.9% NaCl (40 mL) was added to the beaker and mixed by pipetting the sample. A 1-mL sample was removed, serially diluted in 0.9% NaCl, and plated on mannitol salt agar. This protocol allowed for recovery of ~85% of the inoculate. After overnight incubation, the colonies were counted, and survival was reported as the total CFU surviving in the turf. The lower limit of detection of these assays was ~ 1000 (10^3) CFU per milliliter. Because *S. aureus* grows in clusters, each CFU represents clusters of bacteria. Because biofilms are notoriously difficult to disrupt, 400-μL aliquots were directly plated, and survival was scored as plus/minus.

The samples used in this study were placed in beakers to allow for containment of smaller pieces that could be easily removed from the incubator at various points of time (biweekly) after inoculation to determine survival. In addition, placing the turf samples in a beaker allowed sterile saline to be used to recover the bacteria, which gave a better percent recovery than recovering bacteria from the dry surface. The method used in this article for determining survival was empirically developed. To determine percent recovery, the artificial turf was inoculated with a known quantity of bacteria, and then the bacteria were recovered and measured quantitatively. The percent recovery was calculated by dividing the number of recovered CFU by the number of CFU in the inoculums $\times$ 100. It was by doing these types of experiments as part of our study preparation that we determined that placing the pieces of turf samples in beakers and washing them with saline to recover the bacteria gave better recovery than trying to do bacteria recovery without a wash solution.

Preparation of mucin. Pig gastric mucin was resuspended in 0.9% NaCl to a final concentration of 5% and filtered through 0.45-μm PEF membrane filters (Millipore, Billerica, MA). These filters efficiently filter resuspended mucin for use.

All artificial turf samples used in this study were from one distributor. The brand of artificial turf (ProGrass) used in this study was a sand–rubber mixture composition that was the random choice of the authors, and ProGrass had no input into the design or results of this research. The authors have no relationship or affiliation to ProGrass or any other artificial turf supplier.

RESULTS

CA-MRSA survives on artificial turf in a laboratory model. The numbers of viable CA-MRSA slowly declined in the first 2 wk from 10^7 to 10^4 CFU after which point between 10^3 and 10^4 bacteria survived until the experiments were terminated at 20 d (Fig. 1). For one of the experiments,

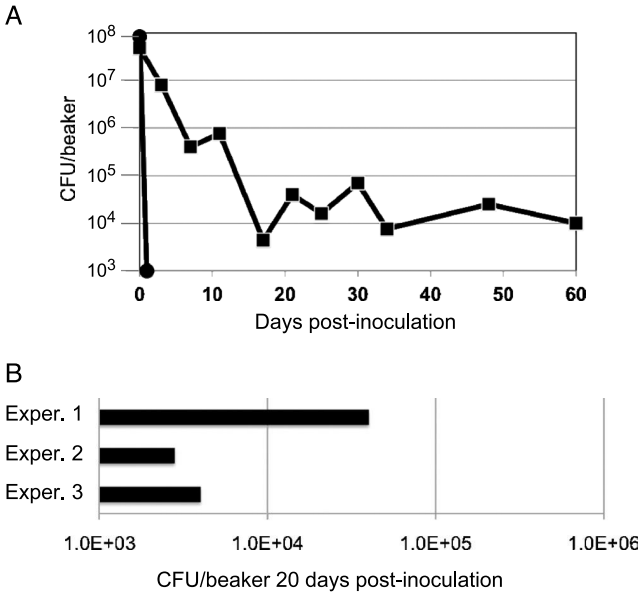

FIGURE 1—CA-MRSA can survive for at least 60 d on turf in the presence of nutrients (*filled squares*). However, in the absence of nutrients (*filled circles*), survival lasts for a few days, suggesting that CA-MRSA survival on turf requires nutrients. The minimum level of detection is 1000 CFU.

in which incubation was extended to 50 d, no further decrease in viability was observed.

Survival is dependent on nutrients. To determine whether a nutrient source was necessary for survival, cells were washed and resuspended in 0.9% NaCl. Under these conditions, CA-MRSA survives less than 3 d.

Biofilm formation does not influence survival. CA-MRSA is capable of forming a biofilm composed of bacteria encased in a matrix (Fig. 3). The matrix composition

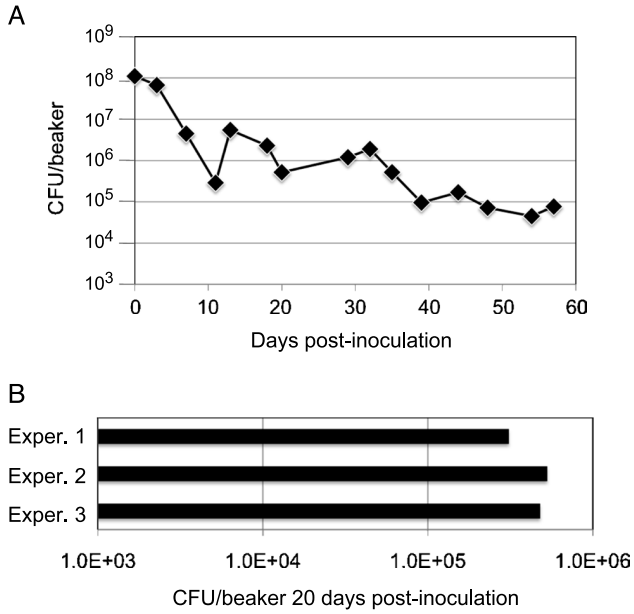

FIGURE 2—CA-MRSA survives in high numbers on turf in the presence of mucin. The minimum level of detection is 1000 CFU.

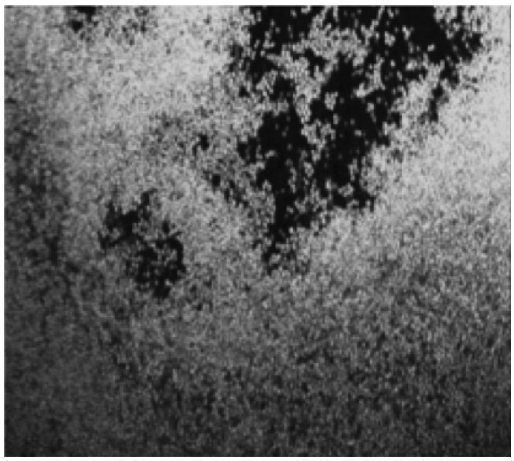

FIGURE 3—CA-MRSA biofilm formation. To ensure proper biofilm formation, biofilms were stained with the DNA stain SYTO 9 and imaged by scanning confocal microscopy using a Leica Sp5. The image shown is representative of the biofilm formation.

is variable and can be composed of polymeric *N*-acetylglucosamine, eDNA, and binding proteins (29,31,38). In some bacterial species, the components of the matrix can serve as sources of sugars and amino acids for carbon, energy, and anabolism in the absence of other nutrient sources (35,39). Biofilm cultures added to the turf with nutrients (BHI medium) survived until the experiment was terminated at 20, 20, and 60 d. Biofilms washed in 0.9% NaCl did not survive past 3 d, suggesting that the matrix did not serve as a nutrient source for CA-MRSA.

Mucin prolongs survival and increases the number of survivors. To determine whether mucin could affect the CA-MRSA survival, washed planktonic cells were resuspended in 5% mucin. For these studies, commercially available preparations of pig gastric mucin were used. The numbers of viable bacteria dropped slowly in the first 2 wk after which time 10^4 and 10^5 bacteria survived for more than 20 d in two independent experiments and more than 60 d in one experiment at which time the experiments were terminated (Fig. 2).

DISCUSSION

Most reported CA-MRSA outbreaks have involved contact sports such as rugby (36), soccer (4,18), wrestling (6,23), basketball (8,37), dance (4), volleyball (1,8,40), and particularly football (2,4–6,8,19,28,33,34), leading many to believe that the primary mode of CA-MRSA transmission involves person-to-person contact both on and off the field, with repeated skin injury (i.e., turf burns and abrasions) and suboptimal hygienic practices (i.e., sharing soap or unlaundered towels with teammates). However, outbreaks reported in noncontact sports including fencing (6), cross-country (1), and weight lifting (8) suggest that transmission may also occur through contaminated fomites such as shared equipment, personal items, or playing surfaces (mats, artificial turf). With the increase in the number of

infections associated with athletic competition, there is growing concern regarding the role of turf surfaces (24).

The goal of this research was to determine whether CA-MRSA can survive on an artificial turf surface. When CA-MRSA was grown in the laboratory, supplied with nutrients, and placed on artificial turf, it survived indefinitely. The survival was bimodal, with high numbers surviving for the first week and lower numbers surviving for longer than 60 d, when the studies were terminated. The bacteria did not continue to grow under these conditions but did survive in numbers high enough that they could hypothetically contaminate a wound. Survival was found to be dependent on nutrients, and when the nutrients were subsequently washed before inoculation on the turf, no survival was noted. Therefore, it was of interest to examine a possible natural nutrient. CA-MRSA survival was supported by mucin (a major component of saliva and nasal secretions). When CA-MRSA were washed of nutrients but mixed with artificial secretions (mucin), the bacteria survived in higher numbers than when studied with nutrients alone without mucin. The ability to use mucin as a nutrient source for survival would have implications for a natural nutrient source of CA-MRSA on artificial turf. MRSA can colonize the naso-oropharynx, and athletes are known to deposit oral secretions onto the field. The survival of CA-MRSA in those secretions could represent a natural reservoir on artificial turf.

One of the major limitations of this study is that, although we have shown that it is possible for CA-MRSA to survive on turf with supplied nutrients in the laboratory for an extended period, we have not shown that this survival will actually occur when subjected to the environmental elements that occur on artificial turf. The survival rates of *S. aureus* on synthetic turf systems and natural grass under different environmental conditions are similar (26). Lower rates of survival have been found outdoors under exposure to ultraviolet light and higher temperatures (26). However, these studies did not take into account nutrients, and decreased survival may have been due to lack of nutrients. In addition, while having no effect on survival under the ideal conditions in these studies, biofilm formation can protect some species of bacteria from UV damage. Although survival in biofilms still required added nutrients, biofilms may confer protection of the CA-MRSA from UV damage. The surface temperatures of actual infill synthetic turf are significantly higher than natural soil surfaces when exposed to sunlight and can reach surface temperatures outdoors that often exceed the temperature range for ideal growth of *S. aureus*, which is 7°C–48°C, with the optimal temperature for growth being 37°C (24). Although *S. aureus* survives well at temperatures as low as 4°C, they may not survive in temperatures as high at 48°C (24). There are generally lower numbers of total microbes in synthetic turf surfaces compared with natural soil due to the lack of a natural soil ecosystem and perhaps due to the presence of zinc and sulfur contained within the artificial turf filler that may inhibit microbial growth (25). Our study did not compare natural soil and artificial turf surfaces and was limited to ideal laboratory conditions. In addition, the strain used in these studies was identified by the CDC as the USA-300 strain, which is suspected to cause most of the CA-MRSA infections. Survival patterns and study results may change if other strains of MRSA species are involved.

Overall, these studies demonstrate the ability of CA-MRSA to survive on artificial turf provided nutrients such as mucin are provided. CA-MRSA has the ability to colonize effectively, even in the absence of antimicrobial pressures and potentially via mechanisms that allow them to compete against other bacterial strains in the nares, the gastrointestinal tract, the perineum, the skin, the axilla, and the oropharynx (11,15). Many studies have looked at nasal colonization in nonathletes (10,22). It has been debated whether nasal colonization with CA-MRSA confers increased risk to the host (10,12,30). Most studies have shown CA-MRSA nasal carriage rates in football teams to be relatively low (16). Whereas most epidemic outbreaks of infection have been studied retrospectively, one prospective study by Creech et al. (9) found surprisingly high rates of nasal and oral colonization in college football and field hockey teams, with increased asymptomatic colonization during the season, which decreased markedly during the off-season; when the entire collegiate student–athlete population was screened at baseline, 11% had nasal colonization with CA-MRSA, with the number increasing to 17% with positive baseline from all sites cultured (nasal, oral, axilla, suspicious skin lesions) (10). Asymptomatic military recruits with initial positive nasal CA-MRSA cultures had a higher incidence of subsequent CA-MRSA infections during the period of training (14). In hospitalized patients, surveillance nasal screening has been shown to be successful in controlling MRSA outbreaks, but similar surveillance strategies with athletic teams have not been shown to be effective in these athletic populations (16).

Even in the absence of direct evidence linking contaminated fomites to the spread of MRSA among athletes, the CDC and the National Collegiate Athletic Association have established guidelines for cleaning and disinfecting playing surfaces, athletic gear, towels, and locker rooms (6,27). Future studies are ongoing to investigate how environmental conditions (humidity, temperature, ultraviolet light) influence the survivability of CA-MRSA in the laboratory model and screening for surviving *S. aureus* and MRSA on actual playing fields.

CONCLUSIONS

In conclusion, this is an experimental study that demonstrates CA-MRSA can survive on artificial turf if adequate nutrients are available to sustain its persistence. Repeated close physical proximity and contact, skin injury, hygienic practice, and exposure to artificial turf are significant CA-MRSA risk factors for athletes who are at risk of infection. With supplied nutrients, CA-MRSA survived on artificial turf. When the study design was repeated with a washed

CA-MRSA sample, survivability was diminished. CA-MRSA requires a nutrient source to survive. The study design repeated with mucin secretions showed CA-MRSA survivability at even greater levels than in the presence of nutrients in a water-based solution. CA-MRSA could potentially use mucin in the form of human secretions as a nutrient source for survival on artificial turf. A relationship between CA-MRSA nasal carriage and subsequent CA-MRSA infection has been established. Nasal and/or oral discharge from athletes may facilitate conditions necessary for survival on artificial, on-field environments. Studies are ongoing to determine whether keratinocytes are able to provide the necessary nutrients for survival and whether environmental conditions (temperature, UV light) may affect survival. Future studies are needed to investigate if artificial turf is related to actual CA-MRSA outbreaks and to see whether this mechanism of CA-MRSA colonization through nasal/oral secretions is a viable hypothesis.

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The results of this study do not constitute endorsement by the American College of Sports Medicine.

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